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AMERICAN JOURNAL OF BOTANY

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ERRATA, VOLUME XVII

- Page 193, 26th line from top. For *Elaeocarpaceae*, read *Elaeocarpaceae*.
 Page 194, 6th line from bottom of table. For *Gessneriaceae*, read *Gesneriaceae*.
 Page 232, 23d line from top, For *diphysun*, read *diphysum*.
 Page 777, 8th line from bottom. For 25, read 15.
 Page 820, 6th line from bottom. For *jasminoides*, read *altissima*.

AMERICAN JOURNAL OF BOTANY

VOL. XVII

JANUARY, 1930

NO. I

BUNCHY-TOP OF ABACÁ OR MANILA HEMP I. A STUDY OF THE CAUSE OF THE DISEASE AND ITS METHOD OF TRANSMISSION¹

GERARDO OFFIMARIA OCFEMIA

(Received for publication July 12, 1929)

INTRODUCTION

Bunchy-top is the most serious disease of abacá, or Manila hemp (*Musa textilis* Née), in the Philippine Islands. This disease destroyed abacá fields in Paete, Laguna Province. On account of bunchy-top, abacá culture has been abandoned in many fields in Cavite Province and the land planted to rice, corn, coconut, and other crops. The disease occurs at the College of Agriculture at Los Baños, Laguna, where many varieties of abacá collected from different parts of the Philippines are grown. The diseased plants in the collection and the seeds from healthy plants furnished an abundance of excellent material for the study of bunchy-top under controlled as well as field conditions.

The present work was started on April 7, 1925, when a specimen of bunchy-top of abacá collected in Silang, Cavite, was brought to the writer at Los Baños by Dr. Otto A. Reinking, pathologist of the United Fruit Company (9). The writer's interest in bunchy-top was stimulated by a conference with Dr. Reinking who called attention to the seriousness of the disease (9). The present paper reports the results of the study of the cause and method of transmission of the bunchy-top of abacá.

THE HOST

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The abacá is indigenous to the Philippine Islands. Its fiber, known in commerce as Manila hemp, is one of the most important materials for export in the Philippines. According to figures of the Bureau of Commerce and Industry in 1928, the total area of land planted to abacá in the Philippines for the year ending June 30, 1926, was 492,050 hectares and the total production was 182,037,295 kilograms (equivalent to 2,878,060 piculs of

¹ Contribution from the Experiment Station of the College of Agriculture, Los Baños, Laguna, Philippine Islands. Published with the approval of the Director.

63.25 kilograms) or an average yield of 470 kilograms a hectare. In 1927 the Philippines exported 148,825,719 kilograms of abacá valued at ₱59,374,258² (average value per ton, ₱398.95). The quantity of abacá exported from the Philippines in 1927 formed 19.08 percent of the total exports and in value it was second only to sugar.

The most important abacá-growing provinces in the Philippines are Albay, Sorsogon, Camarines Norte, Camarines Sur, and Cavite in the island of Luzon; Leyte and Samar in the Visayan Islands; and Davao, Surigao, Misamis, Bukidnon, and Agusan in the island of Mindanao. Until recently, the Philippines had a monopoly of the Manila hemp industry. However abacá has been introduced into other tropical countries and some of them are now said to produce abacá fiber for export. In order to lead in Manila hemp export the Philippines should maintain the present acreage; control bunchy-top, its most important disease; and prevent it from spreading to the other abacá provinces.

THE DISEASE

History, Geographic Distribution, and Economic Importance

The origin and history of the bunchy-top of abacá in the Philippines are not known. The disease is probably an old trouble (9), although abacá farmers in Cavite believe that bunchy-top first broke out in Silang in 1915; it did not, however, become serious until about 1923.

According to Magee (8) bunchy-top of banana, a relative of abacá, has been present in Fiji since about 1885 and the disease was first reported in Australia in 1913. Professor S. F. Ashby, mycologist of the Imperial Bureau of Mycology at Kew, in a letter to the writer dated September 28, 1928, states: "Mr. Magee, who as you know, worked on Bunchy Top in Australia, while returning from Europe this year inspected banana plants in Egypt and Ceylon and found symptoms which are characteristic of the disease in Australia." Magee (8) states that in Australia, abacá and a seedy relative, *Musa banksii*, which is native of north Queensland, are infected by the bunchy-top of banana. He further states that all varieties of bananas in Australia are infected by bunchy-top and their chief and commercial species, *Musa cavendishii*, is very susceptible to the disease.

Although abacá is very seriously infected by bunchy-top in Cavite and Laguna Provinces the writer has not seen the disease on Philippine varieties of banana, *Musa sapientum* and plantain, *Musa paradisiaca*, in the field. The results of experiments to determine the transmissibility of abacá bunchy-top to the important varieties of banana in the Philippines will be reported in the second paper of this series.

Gadd (6) reports that a plot of abacá at the Experiment Station in Peradeniya, Ceylon, was destroyed by a disease with symptoms much like those of bunchy-top of plantains. Magee (8) states that Bryce noted

² ₱1.00 (one peso = 100 centavos) = \$0.50 U. S. currency.

bunchy-top on abacá in Ceylon in 1918 and in October, 1925, definite evidence that the bunchy-top of banana is transmissible to abacá was secured in Australia.

It is beyond doubt that bunchy-top is the most destructive disease of abacá known. Infected plants are very much stunted and rarely produce pseudo-stems a meter long. Although diseased plants do not usually die until after two years, the entire stool becomes absolutely useless and if not removed from the field it is a dangerous source of infection. In a field survey made in January, 1927, Mr. Melanio R. Calinisan found that the disease spread with great rapidity in Cavite. About 95 percent of the abacá fields in that province have been almost completely devastated. Many abacá growers estimate a loss of 80 to 90 percent.

Symptoms

Early Stage of Infection

On the Leaves. In aphid-transmission experiments on abacá bunchy-top under controlled conditions, the writer found that the first noticeable symptom is the presence of indefinite, yellowish-white, chlorotic areas on the blade of the youngest furled leaf. These areas are clearly shown on the margins and lamina of the youngest unfurled leaf. As soon as the leaf which shows the first symptoms is fully expanded, the green portions of the blade on either side of the midrib are usually darker than the green color of the foliage of normal plants. The chlorotic areas are thinner than the rest of the blade and are often retarded in their normal growth. As a result the leaves curl upwards, or tear along the margin. Delicate, thin and transparent membrane-like areas of varying shapes and sizes may develop on the thin chlorotic portions of the youngest leaf either before it unfurls or immediately after. In transmitted light the main veins of the leaves showing the symptoms may be seen as transparent lines (Pl. II *A*, fig. 2, and Pl. III *B* and *C*). These transparent streaks are continuous on the main veins from the midrib to the margin and about one-half of a millimeter in width, but broken into one- to five-millimeter dashes on the secondary veins. On both surfaces of the leaves these dashes appear yellow. If the leaf is furled the streaks sometimes appear water-soaked. The transparency of the primary and sometimes secondary veins was always noted in aphid-transmission of abacá bunchy-top. Dark green streaks, varying from mere dots to lines several millimeters long, or starting from the midrib and disappearing as they reach the yellowed borders (Pl. II *A*, fig. 2) are of great diagnostic value in some varieties of abacá. The dark green lines are about one-half of a millimeter wide and are occasionally present on the midrib, petiole, and leaf blades. Frequently these dark green streaks appear in the more advanced stage of infection, though Magee (8) and the Bunchy-top Investigation Committee (1, 2, 3) in Australia state that the first definite symptom of the bunchy-top on

banana is shown in the leaves by the presence of these irregular dark green dashes which are three-fourths of a millimeter wide and vary from small dots to a line 25 millimeters or more in length. This author further states that at first only a few streaks may be noted but more of them appear later. At first the streaks are present along the secondary veins on the nether surface of the foliage. On (C.A. 10302 Itom ♀ × C.A. 4279 Sinamoro Puti ♂) F₁ abacá seedlings (Pl. IV B, plant 2) on which twenty young aphids had been allowed to feed for forty-eight hours, after having fed previously on diseased plants for about five days, the writer noted that the dark green streaks are also present on the midribs and petioles.

The leaf showing the first symptoms of infection is shorter and narrower than the leaf which was produced before the plant was attacked (Pl. III A and B, and Pl. IV B). The narrowing of the leaf blades sometimes continues down to a few centimeters of the midrib. Owing, perhaps, to its narrowness the leaf showing infection unfurls much more readily than the leaf of a healthy plant. The foliage has a tendency to curl up very conspicuously along its margin (Pl. III B and C) and it is stiffer than the leaf of a healthy abacá.

On the Pseudo-stem. The green portions of the leaf sheaths of infected plants have a darker shade than corresponding tissues in healthy abacá. As soon as an abacá plant is infected, the new or innermost leafsheath begins to become as short as, or shorter than, the one immediately next to it. As the disease progresses the petioles begin to arise from nearly the same plane at the upper end of the pseudo-stem, and as a result the leaves are borne in a more or less rosette arrangement (Pl. I A). The common name of the disease is perhaps due to this conspicuous arrangement of the foliage of infected plants in the field.

On the Roots. In the early stage of infection the root systems are the same in extent and in color as those of the normal plants. This symptom was shown in aphid-transmission experiment 4, sixty-four days after the appearance of the first symptoms of infection (Pl. II C).

Advanced Stage of Infection

On the Leaves. As the disease advances the new leaves produced become progressively narrower and shorter than the ones immediately below and are stiff. In normal abacá plants the expanded youngest leaf is longer and wider than the leaf next to it until the plants begin to flower. The leaves of abacá in the advanced stages of bunchy-top are malformed (Pl. III B). They are much reduced and curled upwards along the margin. The margins are chlorotic and the discoloration extends towards the midrib in the form of irregular diffuse streaks with their long axes parallel with the veins.

In some cases of bunchy-top infection of abacá, the thin chlorotic areas along the margin turn brown and die (Pl. I B) starting from the edge of

the leaf or from any of the thin and transparent membrane-like patches. These changes sometimes continue until the midrib is reached. Often the dead thin chlorotic portions involve the entire length of the youngest furled leaf. During the wet season the dead tissues rot and sometimes this rotting continues downwards, producing heart-rot (10). Under favorable conditions the rotting of the central cylinder may extend as far as the corm.

On the Pseudo-stem. As the youngest leaf sheaths are shorter than the outer older ones the pseudo-stems of bunchy-top abacá are much shorter than those of healthy plants of the same age. The girth at the top of the pseudo-stem of infected plants may be the same as, or greater than, that at the base on account of the shortening of the inner leaf sheaths forcing the bases of the petioles apart (Pl. I *A* and *B*). In healthy abacá plants the pseudo-stems are long, and they taper gracefully towards the upper end because the petioles are set farther apart and the youngest leaf sheath is the longest.

Infected abacá plants may remain alive for more than two years but they gradually become smaller and smaller until finally all of the leaves and leaf sheaths turn brown and die. Thus far, of all observed abacá plants infected with bunchy-top, none have fruited.

On the Roots. In aphid-transmission experiment 4, it was found that about 154 days after the appearance of the first visible symptoms, the roots of the infected abacá seedlings gradually turned dark colored, died, and rotted. Various fungi and bacteria may be found in the rotting roots. The writer believes that the death of the roots in advanced stages of bunchy-top is from starvation, the result, perhaps, of the inability of the diseased plant to furnish them with sufficient organic foods. Magee (8) attributes the rotting of the roots of bananas infected with bunchy-top to a loss of resistance to soil organisms, probably the result of the disorganization of the phloem region of the vascular system. In either case, rotting of the roots occurs as a result rather than as cause of bunchy-top.

Abacá plants attacked by bunchy-top are stimulated to produce numerous undersized suckers, which have small and stiff leaves with curled up margins. On the leaves of the suckers the characteristic symptoms of bunchy-top and the chlorosis are much more pronounced (Pl. II *A*, fig. 2) than on the leaves of plants which were infected from outside sources.

CAUSE OF THE DISEASE

The bunchy-top of abacá in the Philippines has been attributed to various organisms associated with the dead and rotting roots of diseased plants. Hernandez (7) and Teodoro (13), both of the Philippine Bureau of Agriculture at Manila, referred to this malady as bunchy-top or root-rot and stated that the bunchy-top of abacá is associated with a *Pythium*, a *Sphaeronema*, four species of *Fusarium*, and the root-gall nematode, *Hetero-*

dera radiculicola (Greef) Müller. Teodoro and Serrano (14, 15) concluded that the disease which they call bunchy-top or root-rot is caused, in part at least, by a nematode. Ocfemia and Calinisan (12), working at the College of Agriculture at Los Baños with materials collected from Laguna and Cavite Provinces, found that on abacá *Heterodera radiculicola* causes root-knot, a disease entirely different from bunchy-top.

Darnell-Smith (5) found that the bunchy-top of banana is neither due to a "running out" of stock nor to lack of any ordinary soil constituent. The bunchy-top of banana in Australia has been conclusively proved to be due to a virus which is spread by *Pentalonia nigronervosa* (1, 2, 3, 4, 8). According to the Australian workers, bunchy-top falls in the group of virus diseases the transmission of which is restricted to the activity of the aphid vector (1, 2, 8).

The Virus Nature of Bunchy-top of Abacá

The characteristic symptoms of bunchy-top of abacá differ from those of diseases caused by known parasites. The presence of wingless as well as winged forms of the aphid *Pentalonia nigronervosa* Coq.³ which was first noted by the writer on the youngest furled leaves, bases of the petioles, and nether surfaces of the foliage (Pl. II B and Pl. IV A) of abacá infected with bunchy-top at Los Baños in June, 1925 (9), and the relation of the spread of the disease in the field to the distribution of the aphid suggested great similarity to the chlorotic diseases that are transmitted by insects. Believing that the disease is of a virus nature, the writer first determined the relation of *Pentalonia nigronervosa* to the transmission of abacá bunchy-top in June, 1925, and positive results were obtained in August, 1925 (9). In October, 1925, while awaiting the result of the first experiment of bunchy-top transmission through sap-injection and while preparations were being made for further aphid-transmission experiments, Dr. N. B. Mendiola of the Department of Agronomy, College of Agriculture, Los Baños, called the writer's attention to two Australian papers⁴ which were sent to him by Mr. Herbert J. Rumsey, seed and plant merchant of Dundas, New South Wales. These papers contained notes on bunchy-top of banana and referred to the virus nature of the disease. Later, the writer found that in Australia it has been proven conclusively that *Pentalonia nigronervosa* is the vector of banana bunchy-top (1). Dr. Reinking, who recently visited the writer's work on abacá bunchy-top at Los Baños, states that the disease of the abacá in the Philippines is the same as that of the banana in Australia.

³ Identified by Doctor L. B. Uichanco, Department of Entomology, College of Agriculture, Los Baños, to whom thanks are due.

⁴ Nicko's Fruit Journal 5: (No. 12b) 20, September 20, 1925; and The Farmer and Settler and Livestock Breeder's Journal, September 18, 1925.

METHOD OF TRANSMISSION

Experiments with the Aphid *Pentalonia nigronervosa* Coq.*Description of Technique*

In bunchy-top transmission experiments under controlled conditions, potted abacá plants which were grown from seeds produced in the open and from seeds from artificially pollinated plants were used in order to minimize the chance of getting plants with latent stages of bunchy-top. This precaution was of great value because field observations showed that bunchy-topped abacá plants rarely, if ever, fruit (9).

The seeds were germinated in pots of soil which had been sterilized in the autoclave for two hours at 15 pounds' pressure and then allowed to cool. After placing the seeds a centimeter below the surface of the soil the pots were placed out of doors in galvanized iron pans which were fitted on top of wooden benches (Pl. I C). The galvanized iron pans were provided with drainage outlets in the bottom to facilitate the changing of the water. The legs of the wooden benches stood in kerosene cans containing water with a thin layer of crude oil on the top. The water in the pan was five centimeters deep and it was changed frequently during the dry season. On rainy days it was drawn off. It was used in the pans to provide the plants with water and to prevent ants, especially *Ragiolepis longipes* Jerd. and *Dolichoderus bituberculatus* Mayr.⁵ from introducing aphids to the potted abacá seedlings, after the cheese-cloth covers were removed, or from carrying away the insects in transmission experiments. The writer noted that these species of ant were often associated with *Pentalonia nigronervosa* on abacá and banana at Los Baños. The water in the cans in which the legs of the wooden benches stood and that in the metallic pans into which the potted seedlings were placed prevented ants and aphids from unknown sources from interfering with the experiments.

As soon as the seedlings began to emerge the pots were first sprayed with a dilute solution of black-leaf 40 and then covered with cheese cloth (Pl. I C) to prevent any chance of aphid-infestation. When the seedlings had two to three leaves, they were transplanted to sterile soil in 24-centimeter pots. Five seedlings were transplanted to each pot, but as soon as the plants were started they were thinned to one or three. After transplanting, the pots were covered again and placed in the metal pan of water. The method of growing abacá seedlings in pots resting in a pan of water was found very satisfactory during the whole year. During the entire period in which observations were made, the immersion of the pots in five centimeters of water showed no deleterious effects upon the plants.

In the aphid-transmission experiments of bunchy-top, infected abacá plants with an abundance of aphids were collected from the field of the Department of Agronomy, or from the experimental plot of the Department

⁵ Determination by Dr. Uichanco.

of Plant Pathology. From five to twenty or more of the wingless adult aphids were carefully transferred to the youngest leaf or leaf stalk of abacá by using a small camel's hair brush. The aphids were first disturbed before they were picked up one by one with the moistened point of the brush; this care was necessary to avoid breaking the proboscis. No aphids were placed on the control seedlings. After transferring the aphids, the experimental plants and the checks were covered with cheesecloth and the pots were returned to the pan of water on the bench outside of the laboratory. After allowing the aphids to feed on the plants for several days the insects were killed by spraying them with a solution of approximately one cc. of the commercial black-leaf 40 in 1500 cc. of tap water in which about five grams of laundry soap had been dissolved.

Results of Experiments

Experiment 1. On June 30, 1925, twenty infected adult aphids were transferred to each of 30 potted seedlings of variety C. A. 1029 Pinoonan and 30 potted seedlings of variety Itom⁶ which were 40 days old. An equal number of seedlings of the two varieties was used as check. On August 15, 1925, definite symptoms of bunchy-top were shown by 100 percent of the plants on which diseased aphids had been allowed to feed for ten days, while the checks were vigorous and healthy (9).

Experiment 2. In November, 1925, seven-months-old abacá seedlings of variety Inosa and four-months-old seedlings of variety Moro were obtained from the Department of Agronomy. In December, 1925, the young abacá plants were transplanted between rows of coconut seedlings.

On February 2, 1926, eight seedlings of variety Moro and seven of variety Inosa were covered with cheesecloth cages and 20 adult aphids from bunchy-topped abacá were introduced on five of the caged seedlings of each variety. On April 20, 1926, two of the aphid-infested seedlings of Inosa and two of those of Moro were infected with bunchy-top. On June 16, 1926, all of the aphid-infested seedlings were infected, while the checks were healthy.

Experiment 3. On February 1, 1926, 20 aphids from infected plants were transferred to the unfurling leaves of eight 32-day-old potted seedlings of abacá variety C. A. 10302 Itom. Twelve seedlings were used as checks. On February 19, 1926, there was a noticeable difference in color and size of the plants in the checks and those on which aphids were introduced. The latter plants were stunted, and the margins of the leaves were curling up. On February 25, 1926, two of the infected seedlings were dying. On March 6, 1926, all of the seedlings with the aphids were infected. The seedlings in the checks were all healthy.

Experiment 4. On August 17, 1926, five aphids from bunchy-topped abacá were placed on the youngest leaf of each of 20 potted seedlings of

⁶ Of the seeds of six varieties of abacá obtained from Mr. Teofilo F. Novero, of the Department of Agronomy, in April, 1925, only these two varieties were viable.

abacá variety C. A. 4293 Itom which were four months and twelve days old. Four seedlings of the same variety, size, and age were used as checks. After allowing the aphids to feed on the abacá seedlings for nine days the insects on ten of the plants were killed with a solution of black-leaf 40. On the other ten plants the aphids were allowed to remain for 28 days.

At the end of 28 days the youngest leaves of ten of the seedlings, or 50 percent, were narrower and shorter than those of the checks. The leaves had whitish or yellowish streaks and white thin membranous portions on the margin. Twelve days later 18 of the 20 plants used, or 90 percent, showed bunchy-top very distinctly while the checks were healthy.

On October 20, 1926, the root systems of the experimental and check seedlings were examined and the writer noted that they were the same in extent and in color (Pl. II C). On January 20, 1927, the root systems of the experimental seedlings were dark colored and many of the roots were rotted. The writer attributes the rotting of the roots in advanced stages of bunchy-top to the failure of the leaves to supply them with organic foods.

Experiment 5. On August 24, 1926, five aphids from bunchy-topped plants were placed on the youngest leaf of each of nine potted abacá seedlings of variety C. A. 9273 Ihalas which were two months and 22 days old. Six seedlings of the same variety, age, and size were used as checks.

After 20 days, the aphids were killed with black-leaf 40 solution. At this time the chlorotic symptoms, the curling of the margin of the young leaves, and the small, short, and distorted foliage were evident on six of the plants, while the checks were healthy. The same condition of the root systems of the experimental and check plants that was noted in Experiment 4 was also found here.

Experiment 6. On September 2, 1926, 20 adult infected aphids were placed on the unfurled leaves of seven abacá plants of variety Inosa which were one year, three months, and nine days old, and two plants of variety Moro which were one year, one month, and 15 days old. One plant of variety Inosa and three plants of variety Moro were used as checks. The plants were growing in the experimental plot.

On September 14, 1926, the aphids were killed by atomizing them with black-leaf 40 solution. To prevent other aphids from feeding on the plants, spraying with the aphicide was done at frequent intervals.

On October 25, 1926, the yellow streaks, curling of the margin, and small sized foliage were noted on the plants on which there were aphids, while the checks were healthy.

Experiment 7. On February 27, 1928, ten infected adult aphids were transferred to each of nine (Ilayas ♀ × Itom ♂) F₁ seedlings which were 64 days old. The seedlings were growing in pots. An equal number of seedlings was used as checks. The aphids were allowed to feed for eight days. The first symptoms of bunchy-top on the plants on which the infected aphids were allowed to feed were noted on March 20, 1928.

Experiment 8. On April 10, 1928, 40 mature individuals of *Pentalonia nigronervosa* taken from an infected abacá were transferred to the pseudo-stem of a healthy latundan banana, *Musa sapientum* L. var. *cinerea* (Blanco) Teodoro. The outer leaf sheaths of the pseudo-stem of the banana were removed to expose the inner tender tissues. The pseudo-stem was kept in a large battery jar containing a small amount of water to keep the plant fresh. The mouth of the jar was covered with cheese cloth to prevent other insects from molesting the aphids. After about 46 hours, 30 of the aphids were divided equally among three potted (C. A. 9273 Ihalas ♀ × Itom ♂) F₁ seedlings which were three months and 20 days old. An equal number of seedlings of the same variety and age was used as checks. The seedlings on which there were aphids became diseased while the checks remained healthy.

Experiment 9. On April 12, 1928, 50 adult infected individuals of *Pentalonia nigronervosa* were placed on the pseudo-stem of a healthy young latundan banana which was treated as described in Experiment 8. After 48 hours ten of the adult aphids were transferred to each of three young and healthy (C. A. 9273 Ihalas ♀ × Itom ♂) F₁ seedlings. The seedlings infested with the aphids and the control were three months and 22 days old.

In this experiment the abacá seedlings on which the aphids had fed became infected with bunchy-top while the checks remained healthy.

The results of Experiments 8 and 9 seem to show that if infected aphids are allowed to feed on a healthy banana for two days the infective principle is still retained by the insect. Further experiments are in progress to determine how long the aphids can remain infective and whether or not the infective principle is transmissible to their offspring.

Experiment 10. On July 5, 1928, about 150 adult aphids, collected from healthy lacatan banana, *Musa sapientum* var. *lacatan*, at Los Baños were placed on diseased abacá plants in one pot. About 150 young aphids were also placed on three other diseased plants in another pot. The plants were then screened with cheesecloth to allow the aphids to feed undisturbed.

On July 6, 1928, after the aphids had fed on the diseased plants for 24 hours, 20 of the young aphids were transferred to each of two potted (C. A. 10302 Itom ♀ × C. A. 4279 Sinamoro Puti ♂) F₁ seedlings which were 80 days old. Twenty of the adult insects were also transferred to each of two other potted plants of the same variety and age. The plants with the aphids and the checks were covered with cheesecloth. After 48 hours the aphids were killed by spraying the plants with black-leaf 40 solution. Up to September 6, 1928, no symptoms of infection were visible.

Experiment 11. On July 7, 1928, after the young and the adult aphids had fed on the diseased abacá plants for 48 hours, 20 of the young insects were transferred to each of two 81-day-old (C. A. 10302 Itom ♀ × C. A. 4279 Sinamoro Puti ♂) F₁ seedlings. Twenty of the adult insects were

also transferred to each of two seedlings of the same variety and age. At this time there were young aphids borne of the adult insects. Twenty of these were transferred to each of two seedlings of the same variety and age. The pots with the aphids and their corresponding checks were screened to allow the aphids to feed undisturbed. After 48 hours the insects were killed by atomizing them with black-leaf 40 solution. Up to September 6, 1928, the plants were free from bunchy-top.

Experiment 12. On July 10, 1928, after the aphids had been allowed to feed on diseased abacá for about 100 hours, 20 of the remainder of the young aphids which were placed on the diseased abacá on July 5 were transferred to each of two 84-day-old (C. A. 10302 Itom ♀ × C. A. 4279 Sinamoro Puti ♂) F₁ seedlings. Twenty of the offspring of the adult aphids were also placed on each of two seedlings of the same variety and age. The aphids were allowed to feed on the healthy seedlings for 48 hours. The seedlings on which the aphids were placed (Pl. IV B, 2) became infected while the checks were healthy.

Inasmuch as Experiments 10 and 11 did not give positive results further work is under way to determine the shortest time required by healthy *Pentalonia nigronervosa* to obtain the infective principle from the diseased abacá and transmit it to healthy plants. The results of this study will be reported in the second paper of this series.

The writer believes that the results of the aphid-transmission experiments prove conclusively that bunchy-top is readily transmitted by *Pentalonia nigronervosa* from diseased to healthy abacá of different ages. The disease was transmitted to abacá varieties Pinconan, C. A. 10302 and C. A. 4293 Itom, Moro, Inosa, Ihalas, Ilayas, C. A. 4279 Sinamoro Puti, Sinamoro Pula, Santa Cruz, Sinibuyas, Libuton, C. A. 10307 Kinisol, C. A. 1035 Pacoonayan, C. A. 10384 Laguis, (C. A. 9273 Ihalas ♀ × Itom ♂) F₁, (C. A. 10302 Itom ♀ × an unknown variety ♂) F₁, (C. A. 10302 Itom ♀ × Itom ♂) F₁, (C. A. 10302 Itom ♀ × C. A. 4279 Sinamoro Puti ♂) F₁ and (Ilayas ♀ × Itom ♂) F₁.

In the experiments reported in this paper the period of incubation of the disease in variety C. A. 10302 Itom was 18 days; in C. A. 4293 Itom, 28 days; in C. A. 9273 Ihalas, 20 days, and in variety (Ilayas ♀ × Itom ♂) F₁, 21 days. In fast-growing seedlings the period of incubation was shorter than in slow-growing abacá plants.

The Relation of *Pentalonia nigronervosa* Taken from Healthy Abacá and Banana Plants to Bunchy-top Production

On January 20, 1927, numerous aphids taken from healthy banana, *Musa sapientum* var. *lacatan*, were placed on the youngest leaves of potted seedlings of abacá variety C. A. 9273 Ihalas which were 8 months and 26 days old. Aphids were also obtained from healthy buñgulan banana, *Musa sapientum* L. var. *suaveolens* (Blanco) Teodoro, and placed on the

youngest leaves of potted seedlings of abacá var. C. A. 4293 Itom, which were nine months and three days old. The check seedlings were sprayed daily with a dilute solution of black-leaf 40 to prevent aphid-infestation. The experimental and check seedlings were placed in glass chambers for observation until April 19, 1927, a period of nearly three months.

In September, 1927, 25 potted seedlings of each of abacá varieties Santa Cruz, Sinamoro Pula, Sinamoro Puti, Sinibuyas, and Libuton were heavily infested with *Pentalonia nigronervosa* taken from healthy abacá plants. The seedlings varied from six to eight months of age. The checks, consisting of an equal number of seedlings, were sprayed regularly with dilute solution of black-leaf 40. After allowing the aphids to feed on the abacá seedlings for 30 days the aphids were destroyed with black leaf 40. The results of these experiments agree with the conclusion arrived at in Australia that *Pentalonia nigronervosa* which had fed on healthy banana plants cannot produce bunchy-top symptoms (2).

Failure of Transmission through the Soil

In Australia it has been found that although diseased aphids may remain in the soil for a limited period the soil itself is not infected by bunchy-top (2, 8).

The negative results obtained by the writer from experiments conducted from 1925 to 1928, by planting the seedlings in places where abacá stools have died of bunchy-top corroborate this conclusion. Occasional infection of plants used for replacing dead abacá was due to the writer's inability to destroy all the aphids which came from parts of infected plants left in the soil. The results of planting abacá seedlings in soil collected from bunchy-top infected areas of Mendez Nuñez, Cavite, and Los Baños, Laguna, were also negative. The roots of abacá seedlings planted in the pots of soil taken from Mendez Nuñez, Cavite, and Los Baños were attacked by nematodes, *Heterodera radicicola*, producing root-knot (11).

Failure of Transmission by Sap-injection

Experiment 1. On October 12, 1925, three abacá seedlings of unknown source and age were injected with the sap taken from bunchy-top infected plants and two were injected with sterile distilled water as checks. Injection was accomplished by taking a medicine dropper, drawing its point to capillary size, and sterilizing it in the Arnold steamer for 30 minutes. By means of a sterile scalpel the outer sheaths and petioles of infected abacá plants were scratched and the milky sap that oozed out was immediately drawn off with the sterile medicine dropper. The capillary point of the dropper was inserted into the inner side of the petioles of the seedlings in such a way that the sap would drain into the internal tissues.

Experiment 2. On April 15, 1926, two potted seedlings of abacá variety C. A. 10302 Itom, three months and eleven days old, were injected with

the sap taken from infected abacá plants using a $1\frac{1}{2}$ cc. Leuel all-glass hypodermic syringe with B. D. $\frac{5}{8}$ -inch-long steel needle. The syringe was sterilized for 30 minutes in boiling water in a beaker. The juice was taken from the plant in the same manner as in the first experiment. The results of both experiments were negative.

Tests on whether or not knives which had been infected by chopping diseased plants could introduce the virus to healthy plants gave negative results. The results obtained by the writer at Los Baños agree with the findings of Australian workers who report that they failed to reproduce bunchy-top of banana by direct inoculation with the sap from diseased plants (1, 2, 8).

Failure of Transmission with Crushed Tissue

Experiment 1. On May 17, 1926, ten seedlings of abacá variety C. A. 10302 Itom, four months and 13 days old, were used in transmission experiments of bunchy-top, using crushed tissues of diseased plants. Five of the plants were used as checks. The inner portion of the pseudo-stem of a young bunchy-topped abacá was cut into thin slices and ground in a porcelain mortar. Small portions of the crushed tissues were inserted into one-centimeter slits prepared longitudinally with the pseudo-stem using a flamed and cooled scalpel. The slits were made deep enough to reach the heart of the seedlings. In the checks slits were similarly prepared with a sterile scalpel, but nothing was introduced into them.

Experiment 2. On May 17, 1926, another series of experiments was conducted using 46 potted seedlings of variety C. A. 4293 Itom which were 42 days old. Of the 46 seedlings, 38 were inoculated with crushed tissues of diseased plants in the same manner as in the first experiment, and eight seedlings were used as checks. Up to August 12, 1926, the inoculated plants in both experiments were as healthy as the checks.

The Spread of the Aphid Vector in the Field

During the course of the present work the writer noted that the aphid *Pentalonia nigronervosa* which disseminates the disease may be spread in two ways. Observations made at Los Baños in 1925 and 1926 showed that the winged forms of this aphid are not capable of prolonged flight. This method of dissemination in the field seems to be relatively unimportant. The writer noted that the most important agents of the dissemination of the aphids are the ants which live in a symbiotic way with the aphids. At Los Baños the writer found that of the ants transporting *Pentalonia nigronervosa* in the field, *Ragiolepis longipes* Jerd. and *Dolichoderus bituberculatus* Mayr. were the most common. Infective aphids are likely to be carried to other places on the corms, on or between the leaf sheaths, within the intercellular spaces at the cut ends of leaf sheaths, on leaves of living plants, and in soil containing portions of roots and corms of abacá.

THE RELATION OF BUNCHY-TOP OF ABACÁ TO CERTAIN FORMS OF HEART ROT

In a previous paper, the writer (10) reported that in aphid-transmission experiments of bunchy-top, using potted abacá seedlings, some of the infected plants died of heart rot. The same observation was made in the experimental plot of the Department of Plant Pathology and in the field of the Department of Agronomy of the College of Agriculture at Los Baños.

Under the direction of the writer, Mr. Calinisan dug up and examined 500 abacá plants infected with bunchy-top in Mendez Nuñez, Cavite, on January 3, 1927 and found that 13 percent of the diseased plants had early stages of heart rot. He further found that of the additional 200 stools of abacá infected with bunchy-top which he dug up only two and one-half percent had root weevils, *Cosmopolites sordidus* Germar, in the corms.

In January, 1927, 100 abacá plants infected with bunchy-top in the experimental plot of the Department of Plant Pathology at Los Baños were tagged with tin labels. The plants were examined frequently and the occurrence of heart rot was immediately recorded. On April 7, 1928, when all of the tagged plants under observation were dead, the record showed that 17 percent of the plants succumbed to heart rot. The rest of the diseased abacá gradually became smaller and smaller and the leaf sheaths dried up.

On September 28, 1928, another survey made in the abacá field of the Department of Agronomy of the College of Agriculture showed that 52 out of 459 infected plants, or 11.3 percent, had different stages of heart rot. Early in February, 1929 collections of abacá specimens made by the College plant pathology students showed that 33 of the bunchy-topped abacá from Mendez Nuñez, Cavite, and 153 of those from Paete, Laguna, had different stages of heart-rot infection. In heart rot cases following bunchy-top, bacteria and various saprophytic fungi are present in abundance and these seem to hasten the rapid decay of the tender tissues of the heart. Sometimes a foul odor is given off by the rotting central cylinder. It seems probable that this type of heart rot is the same as the bacterial heart rot of abacá reported by Reinking (12) in 1918. The writer does not claim that all heart rot cases in abacá fields are secondary, or final stages of bunchy-top. Observations of plants infected with bunchy-top under controlled conditions and in the fields, however, tend to indicate that from 11 to 17 percent of bunchy-top-infected plants finally die as a result of heart rot.

SUMMARY

1. Bunchy-top is the most serious disease of abacá, or Manila hemp, in the Philippine Islands. The disease destroyed abacá fields in Paete, Laguna Province. In many of the fields in the abacá-growing towns of Cavite Province, abacá culture has been abandoned and the land planted

to rice, corn, coconut, and other crops. This disease occurs on banana in Fiji, Australia, and Ceylon, and is also apparently present on the banana in Egypt. In Australia the disease is said to have been transmitted to abacá and has been reported on this host in Ceylon.

2. The disease first attracted attention in Silang, Cavite, in 1915 but it did not become destructive until about 1923.

3. Abacá plants infected with bunchy-top are very much stunted and very rarely produce pseudo-stems a meter long. Although diseased plants do not usually die until after two years or more, the entire infected stools become absolutely useless and if not removed from the field they are a constant and a dangerous source of infection. The disease spreads very rapidly and in Cavite about 95 percent of the abacá fields have been almost completely devastated. Many farmers estimate a loss of 80 to 90 percent.

4. The first symptoms of bunchy-top on abacá are shown by the presence of chlorotic areas on the margins of the leaves and by transparent streaks and dark green lines along the primary and secondary veins. The dark green streaks vary from mere dots to lines two and a half centimeters or more in length. The green areas of the leaves and of the petioles and leaf sheaths of diseased abacá have a darker shade than those of the normal plants. The leaves gradually become shorter, narrower, stiff, and tend to curl upwards along the margin. The youngest leaves and the subsequent ones produced may be distorted, or even aborted. The leaves of infected plants tend to unfurl earlier than those of healthy plants. The inner leaf sheaths do not grow very much in length and in some cases they are actually shorter than those on the outer portion. As a result there is a conspicuous bunching of the leaves at the upper portion of the pseudo-stem. In advanced stages some of the plants are unable to send out their youngest leaves and these turn brown, die, and rot. The death and rotting of the leaves begin from the chlorotic areas. These are at first almost white, and later turn into thin and transparent dead tissues which finally become brown. In early stages of the disease the roots are normal in color, size, and extent but as the disease advances many of the roots die and rot. The symptoms are more conspicuous on the suckers than on the plants which have received primary field infection.

5. The disease is infectious and in the field it is spread from abacá plant to abacá plant by the aphid *Pentalonia nigronervosa* Coq. Aphids taken from healthy abacá or banana are not capable of causing bunchy-top. Although the winged forms of the aphid can fly short distances, the insects in the field are spread chiefly by ants. The period of incubation of the disease in the varieties of abacá studied varies from 18 to 28 days, depending upon the rate of growth of the plants.

6. Bunchy-top is not transmitted through the soil, by sap injection, or by insertion of crushed diseased tissues.

7. In the present study it was found that from 11 to 17 percent of the abacá plants infected with the bunchy-top disease ultimately die of heart rot.

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EXPLANATION OF PLATES

PLATE I

A. An abacá plant infected with bunchy-top in the field showing the characteristic grouping of the leaves into a more or less rosette arrangement. The specimen was collected from the abacá field of the Department of Agronomy of the College of Agriculture at Los Baños and photographed on January 16, 1926.

B. An advanced stage of bunchy-top infection of abacá showing the drying of the margins of the inner leaves. In some cases plants showing this type of symptoms finally die of heart rot. The specimen was collected from the abacá field of the Department of Agronomy and photographed on January 16, 1926.

C. Portions of wooden benches which were used in germination of abacá seeds and aphid-transmission experiments. The seedlings are covered with cheesecloth to prevent aphid infestation. The covers of three of the pots in the center were removed to show the young abacá plants. Each leg of the bench stands in a dish which contains water with a thin layer of crude oil on top. Photographed July 12, 1927.

PLATE II

A. Back view of small portions of the leaves of (1) a healthy and (2) a diseased abacá, variety Inosa. Note the dark green streaks between the veins of the leaf in (2) as indicated by the arrow-heads. The specimens were collected from suckers of infected stools which were more than two years old. The plant was infected in Experiment 2 of aphid-transmission. The healthy leaf was taken from one of the checks. Photographed in transmitted light on February 6, 1928.

B. A young furled leaf of an abacá sucker (about 30 centimeters from the soil to the base of the petiole of the next youngest leaf) showing a large number of *Pentalonia nigronervosa* Coq. The plant from which this specimen was taken was infected with bunchy-top. The specimen was collected from the abacá field of the Department of Agronomy and photographed on November 21, 1925. About natural size.

C. The root system of one of the checks and that of one of the bunchy-top-infected seedlings (grown from seed) of C. A. 4293 Itom used in Experiment 4 of aphid-transmission, showing that there is no visible difference in size, color, and development of individual roots in healthy and in diseased abacá seedlings 64 days after the appearance of the first symptoms of infection. Photographed on October 20, 1926.

About 154 days after the first symptoms have appeared the root systems of the remaining diseased plants were dark colored and many of them were rotted.

PLATE III

A. One of the check abacá plants var. C. A. 9273 Ihalas (grown from seed) used in Experiment 5 of aphid-transmission, showing clean and wide open leaves which are larger in size than those of diseased plants of the same age. This photograph was reduced more than that shown in B of this plate.

B. The aerial part of one of the abacá seedlings (grown from seed) variety C. A. 9273 Ihalas used in Experiment 5 of aphid-transmission, when they were two months and 22 days old. Five adult aphids were placed on each plant and the insects were allowed to feed for several days. The seedlings were infected 20 days after the aphids were allowed to feed on the seedlings. This photograph is more enlarged than A to show the yellowish streaks on the youngest leaves marked (x) and the much reduced and distorted foliage indicated by the arrow-head.

C. One of the young and reduced leaves of the infected plant shown in B of this plate. The leaf shows the curling along the margin and the yellow streaks in the regions of the veins. A, B, and C were photographed on October 20, 1926.

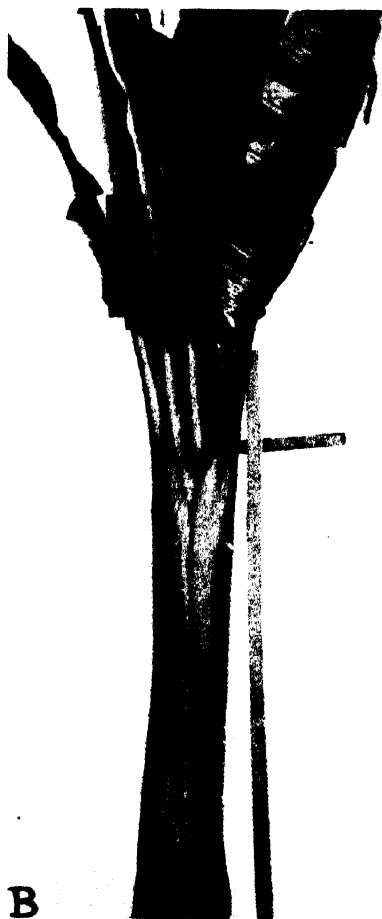
PLATE IV

A. Colonies of *Pentalonia nigronervosa* Coq. on the petioles and lower surfaces of the leaves of a young potted abacá plant grown from seeds which were taken from Indang, Cavite. In the field the aphids form the same type of colonies on abacá suckers. Photographed on September 21, 1928.

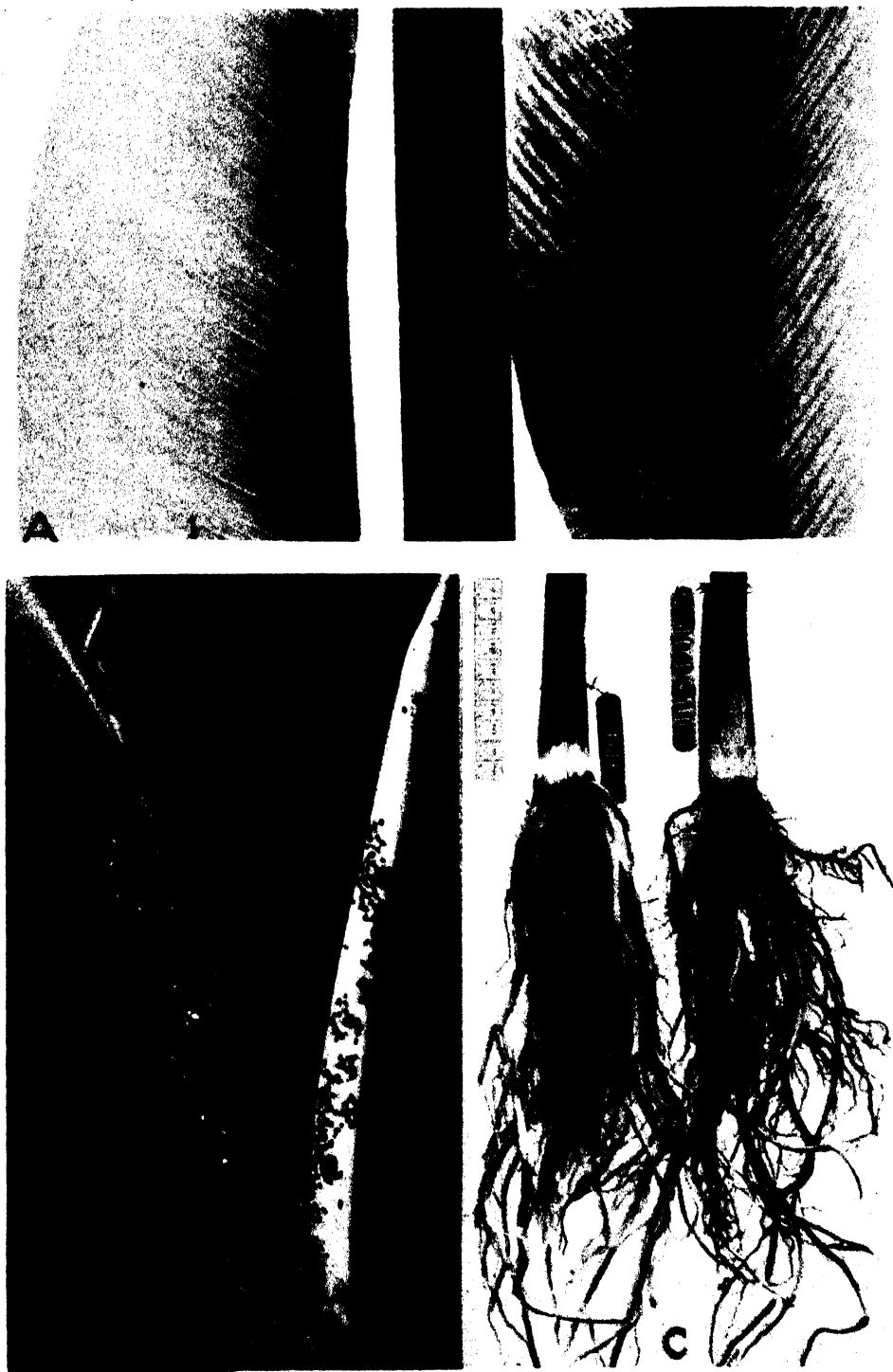
B. In (1) is shown a 158-day-old abacá seedling var. (C. A. 10302 Itom ♀ × C. A. 4279 Sinamoro Puti ♂) F₁ used as check in Experiment 12 of aphid-transmission. In (2) an abacá seedling of the same variety and age showing typical symptoms of the infectious bunchy-top. About 150 adult aphids were placed on a diseased abacá seedling on July 5,

1928. Twenty of the offspring of the adult insects were placed on each of two 84-day-old seedlings on July 10, 1928. After allowing the aphids to feed on the plants for 48 hours, the insects were destroyed with dilute solution of black-leaf 40. Note the appearance of plant 1 and plant 2 and compare the symptoms of plant 2 with those of the infected plant in Plate III, *B*. Photographed on September 21, 1928.

Plate I, *A*, *B*, and *C*, Plate II, *A* and *B*, and Plate IV, *A* and *B*, from photographs by the Photographic Division, Department of Agronomy, College of Agriculture, Los Baños. The rest from photographs by the Philippine Bureau of Science, Manila.



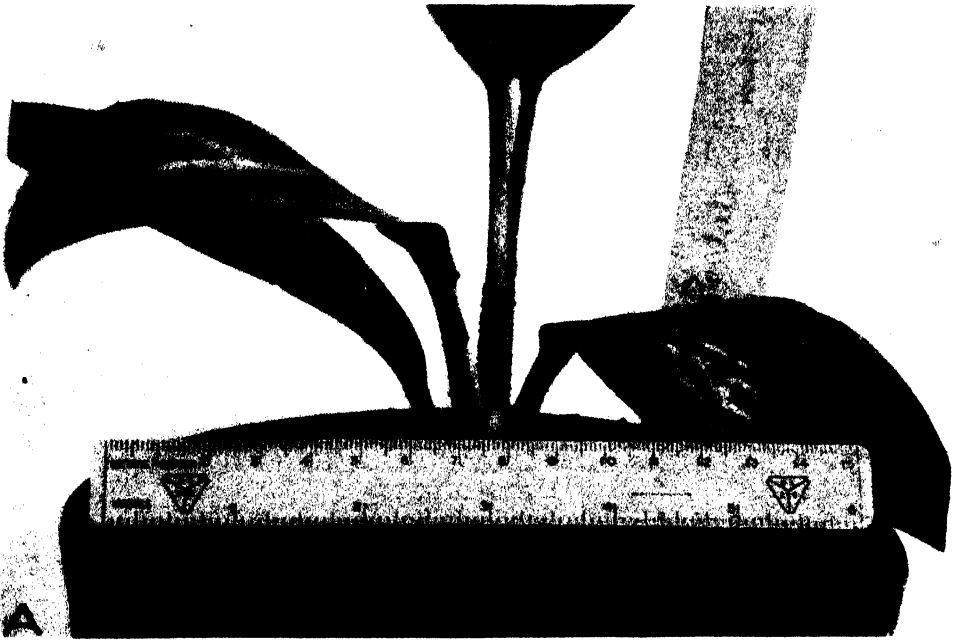
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THE MORPHOLOGY OF *OXYMITRA ANDROGYNA*

JESSE Q. SEALEY

(Received for publication July 15, 1929)

Because of its intermediate position between the Ricciaceae and the lower Marchantiaceae, the genus *Oxymitra* (*Tessellina*) is of peculiar interest. The thallus has the general characters of the latter group, while the simple type of sporogonium resembles very closely that of *Riccia*. The sporophyte characters have been in this case regarded as more significant and the genus has been placed in the Ricciaceae. Schiffner (17) divides the family Ricciaceae into three genera, *Riccia*, *Ricciocarpus*, and *Tessellina*, which division is also followed by M. A. Howe (11).

Until within very recent years only one species of *Oxymitra* has been recognized, *O. paleacea* Bisch. Its known distribution was limited to the Mediterranean region of Europe and Africa, until Balansa, in the early eighties, reported it from Paraguay. About twenty years later Stephani reported it from Brazil. In 1914 Dr. M. S. Young, of the University of Texas, collected specimens of *Oxymitra* near Austin, Texas. This material was first thought to be identical with the European *Oxymitra paleacea*. M. A. Howe (10), from a study of the Texas plant, found it to be monoecious while the *Oxymitra* previously reported seems to be strictly dioecious. The spores of the Texas plant were found to average considerably larger than those from plants reported elsewhere. They range from 125 to 175 microns in diameter, while those of the European form vary from 104 to 140 microns. The radial wall of the epidermal cells bounding the pores or stomata seemed to be more strongly and abruptly thickened than in the European species. The monoecious character together with these other peculiarities of the Texan plant were considered sufficient to justify its segregation into a separate species. The new species was described as *Oxymitra androgyna* by Howe (10).

The beginning of exact morphological knowledge of *Oxymitra* dates from Leitgeb's work in 1879 (12). He made a rather careful study of the genus, and described in considerable detail the general morphological characteristics of both thallus and sporophyte. He emphasized its intermediate position, and pointed out both the resemblance of its thallus to that of the Marchantias and its pronounced *Riccia*-like sporophyte, which he considered barely a step in advance of the true *Riccia* type. Considering that he was limited to free-hand sections he was able to make some very accurate observations concerning the sporogonium. He observed a more or less

imperfectly differentiated peripheral wall, with possible indications of sterile sporogenous cells, particularly near the apex and base of the capsule.

Howe (9, 10) seems to have published the only work upon *Oxymitra* since Leitgeb's classic study in 1879. The abundance of material to be found in the Austin vicinity, together with the desirability of verifying the work of Leitgeb by using the more modern technique, particularly the paraffin method, and of comparing the morphology of the Texan species with that of *O. paleacea* as described by Leitgeb, has led to the present study.

HABIT OF GROWTH

Oxymitra is of very common occurrence in the vicinity of Austin. Although this plant is perennial, it is conspicuous only during the late fall and the winter months. It has been collected from time to time by Dr. F. McAllister and others from a number of localities in this section of the state, being reported from as far east as College Station near Bryan, Texas, and as far west as Marble Falls, Texas.

It grows in a limited variety of soils but is found more abundantly in the sandy loam of the upland post-oak woods, growing in open spots along with such plants as *Selaginella Riddellii*, *Asterella echinella*, *Asterella tenella*, and the *Riccias*.

The thalli are 1-3 times dichotomous, usually more or less loosely gregarious, and seldom attain a typical rosette-like arrangement, due to the growth cycles and the decay of the basal portion of the thallus. The plants are especially characterized by the large white, ventral scales, which project some distance beyond the margins of the thallus, forming a conspicuous fringe about the plant. During dry periods the dorsal surfaces of the thallus fold together and the scales from opposite sides, by rolling inward, tend to overlap, thus probably serving to prevent in a measure the very rapid and excessive drying out of the spongy thallus. The plants thus folded up are very inconspicuous and within two or three days at the beginning of a dry period the plants will practically disappear from view. The plants are in this way enabled to survive drought, and will open up within a very short time after a rain. Thus plants collected during dry periods and to all appearances entirely dead were revived within a few hours when furnished with proper moisture. In keeping with this habit of the plant it seems, during the winter, to go through more or less definite growth cycles, corresponding to the periods of precipitation followed by periods of drying out when the surface of the soil becomes particularly dry. Thus plants very often produce their sex organs and sporophytes in interrupted series. At the beginning of a period particularly favorable to growth the thallus grows very rapidly until it attains the length of some 6 or 8 mm., after which it produces a series of sex organs and sporophytes. As these mature the thallus grows very slowly. If then there follows another period of precipitation especially favorable for growth, there will be another rapid

vegetative development and the production of a second series of sporophytes. In this manner the same thallus may produce several crops of spores during the same season. The first crop in the fall is, however, nearly always much the largest.

The appearance of the archegonia and developing sporophytes is closely followed by the appearance of the cone-like involucre which arise from the median sulcus and project above the level of the thallus, developing to considerable size and becoming fairly conspicuous as the sporophytes mature. The involucre is practically the same shade of green as the remainder of the thallus until the sporophyte reaches maturity, after which they often become brownish in color, finally becoming almost black. Plants observed early in October had begun to show the appearance of the cone-like involucre of the archegonia and by the middle of November the first practically mature sporophytes were observed.

GENERAL MORPHOLOGY

Material for this study was collected and observed at short intervals beginning late in September and continuing through the middle of January following, of the years 1927 and 1928. A variety of fixing agents were employed, but the medium and the weak chromo-acetic solutions (Schaffner's) seem to give uniformly good results. A simple air pump, as described by Osterhout (16), was used to hasten the penetration of the fixing solutions. The paraffin method was used in preparing and sectioning the material. Both Heidenhain's iron-alum haematoxylin and Flemming's triple stains were used.

Difficulty in cutting sections due to the tough ventral scales was somewhat overcome by trimming off the edges of the thallus before fixing.

The thallus of *Oxymitra* varies considerably in size and proportions, depending upon the conditions under which it grows. Its development seems to follow in the main that of other Ricciaceae as outlined by Campbell (4). The dorsal or green spongy tissue of the thallus constitutes from one-third to one-half of its total thickness, and is made up of irregular plates of chlorophyll-bearing tissue, usually one cell in thickness, inclosing long tube-like air chambers, which open upon the upper surface through elevated pores. These air pores are surrounded by five or six radially arranged cells with greatly thickened radial walls. These pores with their thick-walled border cells suggest, in general appearance, blunt pointed stars in the upper epidermis.

Miss Hirsh (8), in her work on the origin of the air chambers in the Ricciaceae, came to the conclusion that there are two methods of air chamber development in the Riccias, one by internal cleavage, resulting in irregular air spaces separated by plates of cells usually one layer thick, the other by upward growth of filaments at right angles to the surface of the thallus, forming narrow chambers or canals as in *Riccia Frostii* Aust. This is in

general agreement with the accounts of both Leitgeb and Campbell. The air chambers in *Oxymitra* seem to originate according to the first method.

The tissue of the ventral part of the thallus is compact, with but few intercellular spaces. It gives rise to the rhizoids and ventral scales. The latter arise in two series on the upper lateral edges of the compact tissue of the thallus and are, according to Leitgeb, homologous with the walls separating the air chambers.

The thallus is marked by a deep and rather conspicuous longitudinal median groove extending from the growing points. In fruiting material the antheridial involucre and cone-like archegonial involucre are produced along this sulcus, and may be seen rising above the surface of the thallus. The sex organs and sporophytes are produced thus in groups along this median sulcus. *Oxymitra androgyna* is monoecious and very often scattered antheridia are found laterally disposed to the larger groups of archegonia or developing sporophytes. Occasionally, on the other hand, the antheridia are produced in distinct aggregations. There seems to be no such localization of sex organs as Garber (7) has described for *Ricciocarpus natans* in which the antheridia are borne on the older tissue near the base of the thallus and the archegonia nearer the growing point.

As with other Ricciaceae the sex organs arise just back of the growing point, often in a dorsal furrow. The development of the antheridium conforms in general to the *Riccia* type as described by Garber (7), Campbell (4), and Lewis (14). The antheridium initial may be distinguished from the adjacent cells by its dense protoplasmic contents. The spermatogenous cells are recognizable early, by the fact of their dense protoplasmic contents as compared to the more vacuolate cells of the periphery. The cells of the spermatogenous tissue in young antheridia have rather finely granular cytoplasm, and large nuclei.

The antheridium is usually a more or less oval body with conical apex, although some are observed to be almost perfectly oval in outline (Pl. V, figs. 3, 4). As the antheridium initial divides the adjacent cells also undergo division forming a mass of cells which very soon encloses the young antheridium, finally developing into the slender involucre which envelops the antheridium at maturity (figs. 3, 4, 1).

In their earlier stages the archegonia seem to follow the line of development outlined by Garber (7), Campbell (4), and Lewis (14) for the *Riccias*. The neck canal cells number four or five (figs. 7, 8, 9). The egg almost completely fills the archegonium, and at maturity its protoplasm is rather dense, being only very slightly vacuolate (fig. 10). At the time of fertilization numerous deeply staining bodies which are probably plastids may be observed in the egg cell, particularly near its periphery (fig. 11). At about the time of fertilization the basal cells of the young archegonium, which lie beneath the egg, divide to form a rather prominent tissue of small cells which has the effect of lifting the spore capsule somewhat above the storage

tissue beneath (figs. 12, 13). These cells may possibly digest the abundant starch from the cells beneath so that it is made available for the growing sporophyte, thus functioning as Garber suggests in the case of *Ricciocarpus*, as an equivalent of the foot of the sporophyte in higher forms.

As the initial cells of the archegonium divide, the adjacent cells also undergo division, forming a number of irregular plates of cells around the developing archegonium. These plates constitute the beginning of the cone-shaped involucre which keeps pace with the developing neck cells of the archegonium so that it is never more than very slightly exerted from the surrounding tissue (figs. 8, 9). As these plates of cells lengthen, air chambers are formed by the internal cleavage of the lateral walls, in the same way as the air chambers of the chlorophyll tissue of the thallus are formed. This conical envelop or involucre attains considerable size and has a definite epidermis with the characteristic air pores (fig. 1). This specialized structure, as pointed out by Cavers (5), suggests the more specialized involucre and sporangiophores of higher forms.

THE SPOROPHYTE

Since *Oxymitra* occupies an intermediate position between the Riccias and the lower Marchantiaceae, we might expect to find in the form and development of its sporophyte some deviations and indications of growing complexity over the lower forms. For this reason the development of its sporophyte is followed with some interest.

Leitgeb (13) made a very careful and accurate study of *Oxymitra paleacea* and although limited to the technique of free-hand sections, he followed the development of the sporophyte rather accurately. He observed that the developing embryos are of two general forms, spherical or cone-shaped, due probably to the size and shape of the venter cavity in which they are formed. The cone-shaped embryos he found to be more common than the spherical forms. They can be recognized in stages as late as those showing the beginning of the separation of the sporogenous cells.

Leitgeb observed what he regarded as a further deviation from the ordinary *Riccia* type in the late differentiation of the peripheral layer of the sporophyte. As a result of this late differentiation, the wall layer does not at any time stand out as a sharply defined single layer. Even in capsules in which the inner cells have begun to round up, it is not possible to distinguish the cells of the capsule wall from the spore mother cells. He observed that certain cells of the peripheral layer are considerably elongated in a radial direction, extending far beyond the neighboring peripheral cells. Sometimes instead of one long cell, several small cells similarly arranged were observed, which on account of their size were nevertheless to be interpreted as wall cells. This, according to Leitgeb, indicates that it is not possible in certain stages of development to recognize the later function of a cell simply from its position and size. He observed furthermore that

there is no ground for designating all peripheral cells as wall cells nor all inner lying cells as spore mother cells. By staining these cells with iodine he found that the contents of the cells at the periphery stain blue, while the inner cells remain yellowish. Frequently there were several radially arranged deeper lying cells whose contents stained blue, indicating starch content.

The first division of the fertilized egg is usually oblique. The second division is perpendicular to the first (fig. 12). Subsequent divisions do not seem to be absolutely uniform but the embryo gradually develops, apparently by irregular cell division, into a rounded embryo, six or seven cells across at the largest diameter (figs. 12, 13). My observations are in agreement with those of Leitgeb in regard to the general shape of the young sporophytes. The majority of those observed were somewhat flattened at the base and pointed at the apex, particularly during the younger stages (fig. 13). This somewhat pyramidal shape may be retained until as late as the formation of the spore tetrads. Divisions cease in the sporophytic cells when the sporogonium is about one-third its mature size. At this stage it is not possible to differentiate the cells of the capsule wall from the spore mother cells, although the nuclei of most of the peripheral cells seem relatively much smaller than those of the inner cells (fig. 13). Elongating cells similar to those referred to by Leitgeb may be observed at this stage.

At about the time of fertilization the wall cells of the venter divide to form two layers. They do not undergo further division but enlarge to accomodate the growing sporophyte. The cells of the inner layer enlarge to keep pace with the increasing circumference but do not thicken radially, while the cells of the outer layer enlarge in all dimensions (figs. 13, 14, 15). The dimensions of the cells of the outer layer at the time of maturity of the sporophyte are three or four times as great as at the time of their last division. As the spore mother cells develop the inner of the two layers of venter cells is considerably crushed and later absorbed, while the outer layer remains intact until after the complete maturity of the spores (figs. 14, 18). Numerous chloroplasts may be observed in the cells of this layer.

As the cells of the sporophyte enlarge the calyptra and wall layer enlarge faster than the interior cells, giving rise to intercellular spaces on the interior. This allows the spore mother cells to separate from one another and to **round up**. These intercellular spaces seem to appear first in the apical region, between the outer layer of cells and the **spore mother cells** (fig. 14). Most of the cells of the outer layer at the **apex** are somewhat elongated and horizontally arranged, while in many **cases** those at the base are vertically arranged (fig. 15).

At the time when the spore mother cells begin to **round up**, the external cells of the sporogonium are plump and show no signs ~~whatever~~ of collapse, and in the density of their protoplasm, cannot be distinguished from the spore mother cells (fig. 15). At this stage the nuclei of the sporophyte

cells are of two distinct types. One type with relatively small, dense nuclei occurring in the peripheral and smaller adjacent interior cells and the other type with very large usually faintly staining nuclei found in most of the interior cells and very rarely in one of the peripheral cells.

This irregularity in the character of the cells of the sterile wall layer becomes more evident as the spore mother cells become finally differentiated. At this stage most of the cells of the external layer undergo rapid shrinkage and distortion while most of the interior cells round up and come to lie free in the cavity of the sporogonium (fig. 16). Occasionally cells lying adjacent to the inner layer of the venter show little or no disintegration and tend to round up, suggesting that they are about to function as spore mother cells (figs. 16, 17). There are also frequent cases in which at least two layers of disintegrating cells may be seen at the periphery (fig. 17). This sterile peripheral layer of the sporophyte is thus seen to vary considerably, ranging from those cases where it seems to be entirely lacking and the fertile cells lie in contact with the calyptra, to cases in which it is at least two cells in thickness.

Very soon after the spore mother cells begin to round up there accumulates between the rounded cells a dense and stainable mucilaginous material which seems to be derived from the now very rapidly disintegrating wall cells (fig. 17). This material is possibly nutritive in nature and may furnish an important part of the food necessary for the rapid increase in size of the spore mother cells and development of the spore tetrads. Garber (7) and Lewis (14) have both observed similar mucilaginous material in *Riccia*, and believe that it is derived largely from an excess of food material found in the adjacent gametophyte cells. McAllister (15) observed a similar mucilaginous accumulation in *Riccia Curtisii*, but believed that it could not have come from the adjoining cells, since no excess food could be identified in them, nor was there any marked change in these cells upon the appearance of the mucilaginous material. The amount of colloid present, he believed, could be accounted for by the hydrolysis of the walls of the spore mother cells together with the disintegration of the wall layer of cells. This seems to be the explanation in *O. androgyna* since it alone will correlate the simultaneous appearance of the mucilaginous material with the very rapid disintegration of the wall cells, which is also accompanied by the very rapid increase in size of the spore mother cells. The mucilaginous material persists for some time, finally disappearing at about the time of the formation of the true spore wall in the spore tetrad.

The inner layer of calyptra cells has been considerably flattened by the growing sporophyte, yet the sporogonium walls are the first to disintegrate. The breaking down of the wall cells begins soon after the spore mother cells begin to round up. Certain of the cells lying within the wall layer undergo disintegration along with the sporogonium wall cells, thus possibly functioning as incipient elater cells (figs. 16, 17, 18). Remnants of the

inner calyptra cells, and of occasional sporogonium wall cells, may be observed even after the spores are practically mature (fig. 18).

DISCUSSION AND SUMMARY

It will be seen from the above that *Oxymitra androgyna* does not differ notably in morphological details from *O. paleacea*. The monoecious condition and the size of the spores as reported by Howe seem to constitute the most usable characters by which the two species may be distinguished.

The habit of rolling up, with the thick ventral scales as a protective outer covering, seems to delay drying until the active cells have become sufficiently dormant to escape serious injury. At any rate *Oxymitra androgyna* lives throughout the year with little more change than occurs in the drought-resisting Jungermanniales. It thus ranks with the few strictly perennial Marchantiales.

The sporophyte diverges from the current descriptions of the *Riccia* sporophyte in certain details which are probably of minor importance. The absorption of the sterile cells of the sporophyte as the mother cells and spores mature is distinctly *Riccia*-like. Deviations occur, however, which may be of some significance. The sterile wall layer of the sporophyte shows considerable variation in thickness. Cases were observed in which the rounded spore mother cell lies in contact with the cells of calyptra. Parts of the sterile wall layer are often two layers of cells in thickness. The sterile and fertile cells cannot therefore be distinguished by their position alone. Most of the cells of the external wall layer have small, dense nuclei and most of the cells of the interior have large, loose nuclei. This seems to be the best criterion for the identification of the two kinds of cells before the rounding up of the spore mother cells immediately preceding the reduction divisions. There is no such distinct difference in the staining reaction of the contents of the fertile and the sterile cells as has been described by Leitgeb (12) for *O. paleacea*. Neither with Flemming's triple stain nor with iodine could such conspicuous accumulations of starch be detected as he described as occurring in the sterile cells of the sporophyte. There is at no time in *O. androgyna* a clearly defined sporogonium wall as has been figured for *Ricciocarpus natans* (7, 14) for *Riccia crystallina* (14), for *Riccia glauca* (1), and for *Riccia Frostii* (3). The distortion and disintegration of the sterile cells is so rapid that a distinct and definite wall layer cannot be identified. In this respect *Oxymitra* resembles *Riccia Curtisii* very closely (15).

It is doubtful whether the evidence justifies the interpretation of any inner sterile cells in the sporophyte of *O. androgyna* as elater cells. It would seem more reasonable to interpret all of the cells of the sterile peripheral layer as elaters since they are wholly nutritive in function.

There is no evidence of increase of sterile tissue at the base of the sporogonium to form a rudimentary foot. There are as a matter of fact

fewer signs of digestion of archegonial tissue in this region than in the lateral and apical regions.

This work has been done under the direction of Dr. F. McAllister, to whom the author wishes to acknowledge his indebtedness for advice and criticism.

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EXPLANATION OF PLATE V

All drawings were made with the aid of a camera lucida. The magnification is approximately as follows: FIGS. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, $\times 240$; FIGS. 13, 14, 15, 16, 17, 18, 19, $\times 120$.

FIG. 1. A portion of a section of a thallus, showing sporophyte, antheridium, and part of an archegonium.

FIGS. 2-6. Stages in the development of the antheridium, showing both the oval and pointed types.

FIG. 7. Early stage in development of archegonium.

FIG. 8. Young archegonium, becoming submerged by outgrowth of surrounding cells.

FIG. 9. Archegonium, not quite mature, showing the first air chambers, formed between the plates of involucre cells.

FIG. 10. Mature archegonium, showing unfertilized egg.

FIG. 11. Archegonium ready for fertilization; dark staining bodies, probably starch grains, near periphery of egg.

FIG. 12. Four-celled embryo in form of quadrant, showing oblique division. Venter wall cells and those at the base undergoing division.

FIG. 13. Later stage in the development of the sporophyte. A conspicuous tissue of cells has developed at the base.

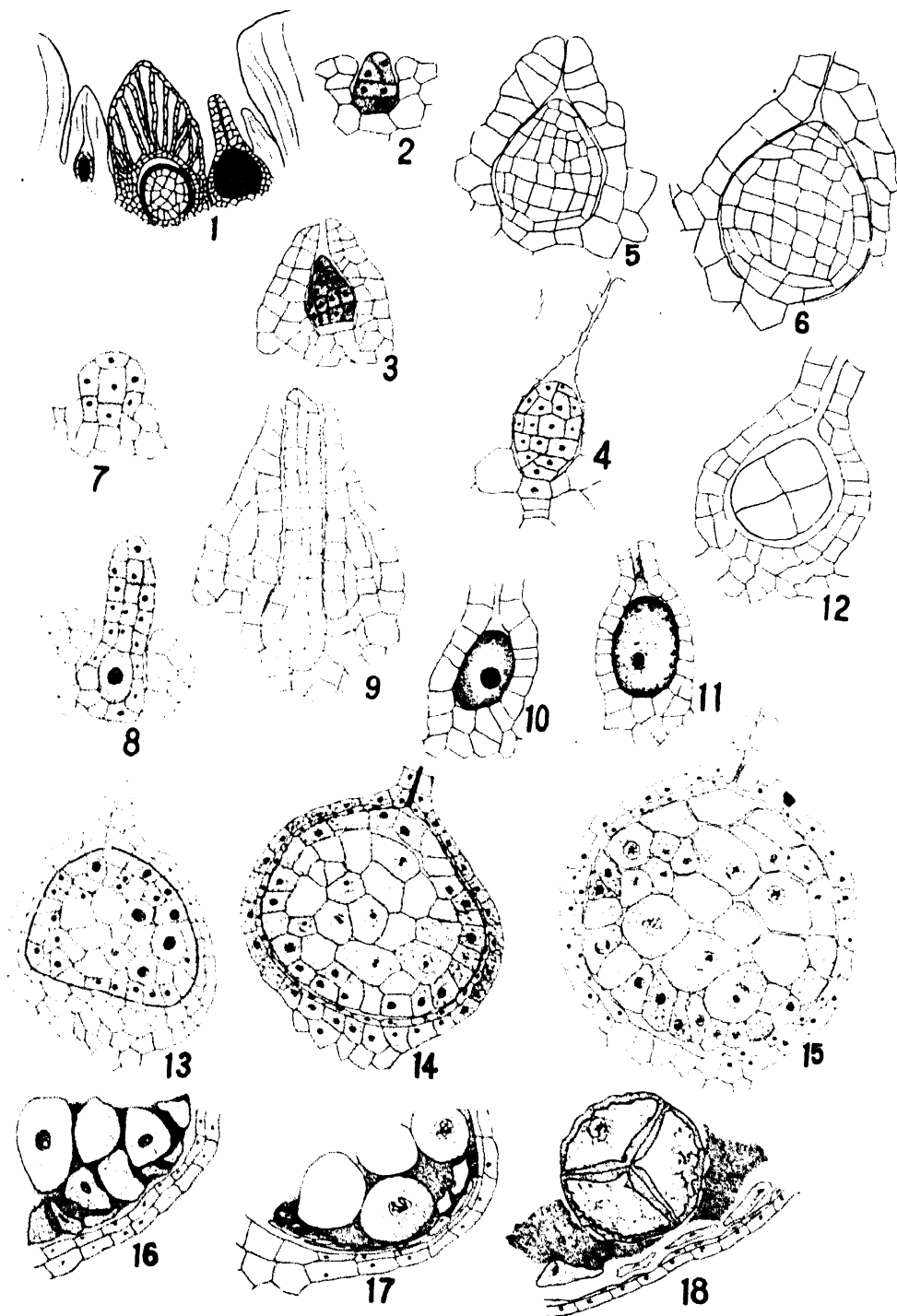
FIG. 14. Sporophyte about half grown. Inner layer of calyptra cells much thinner than the outer layer.

FIG. 15. Spore mother cells beginning to round up, wall cells still plump and intact. Spore mother cells with large light nuclei.

FIG. 16. Accumulation of mucilaginous material between spore mother cells. Both interior and capsule wall cells showing evidence of disintegration.

FIG. 17. Spore mother cells completely rounded up. Remnants of both interior and capsule wall cells being disintegrated.

FIG. 18. Maturing spore, considerably increased in size, showing wall formation. Remnants of wall cells and mucilaginous material still visible. Inner layer of calyptra very nearly gone.



SEALEY: OXYMITRA

NUCLEAR AND CYTOPLASMIC DIVISION IN THE MICRO- SPOROGENESIS OF ALFALFA

R. G. REEVES

(Received for publication July 30, 1929)

A knowledge of the reproductive processes of a plant as important as alfalfa (*Medicago sativa* L.) should be of interest and value to both the botanist and the plant breeder, and careful cytological studies of these processes should yield useful information in the solution of such problems as failure to set seed. The present investigation was undertaken to increase our knowledge of reproduction in this species, about which but little is known at present.

MATERIAL AND METHODS

The material investigated was limited to *Medicago sativa* L., and representative plants of the common and variegated groups were studied. Grimm alfalfa was used throughout as the variegated type. Some of the material was collected from the experimental plots of the Department of Farm Crops, Iowa State College, and the remainder was grown in the greenhouse. Careful records were kept in order to recognize any possible differences which might have arisen on account of the different conditions under which the plants were grown. Observations were also made to determine any cytological differences between the common and variegated types. The two types show no consistent differences, however, and all of the data presented will therefore be applicable to either type.

A number of fixing reagents were employed. A modification of the special chromo-acetic-osmic solution was found very good throughout. It consists of chromic acid 3 g., glacial acetic 2 cc., osmic 2 cc., and distilled water 95 cc. Some good preparations were secured by fixing in Licent's fluid, also. Benda's and Bouin's fluids were tried but did not prove successful.

In order to verify the chromosome counts made in the pollen mother cells, root-tips were killed in Licent's fluid, prepared in the usual way, and examined.

Heidenhain's haematoxylin and Flemming's triple stain were used in studying mitotic figures. For the investigation of cell walls and cytokinesis, Delafield's haematoxylin and Flemming's triple stain were used with fixed material, and resorcin blue (lachmoid) and aniline blue with fresh material.

OBSERVATIONS

Development of the Anther

The very young anther is a four-lobed mass of meristematic cells. A hypodermal column of one or two cells in thickness, the archesporium, soon differentiates. These cells give rise to the primary parietal cells and the primary sporogenous cells by the formation of periclinal walls. The primary parietal cells then divide by another periclinal division and the outer daughter cells redivide in the same manner. The two middle layers become parietal tissue and the inner one the tapetum. The anther wall consists, therefore, of an epidermal layer, two middle layers, and a layer of tapetum (Pl. VI, fig. 1). The primary sporogenous cells divide a few times and produce a column of pollen mother cells.

The tapetum differentiates rather late and its cells remain uninucleate. The outer parietal cells become greatly elongated radially, differentiating into an endothecium. While the pollen is developing, the tapetum and the inner layer of parietal tissue disorganize and their contents are consumed by the developing pollen. When the pollen is fully mature the wall of the locule consists of only the epidermis and the endothecium.

The Heterotypic Nuclear Division

When the pollen mother cells enter the prophase of the first meiotic division their protoplasts are angular and are pressed firmly together (fig. 1). A surprising amount of variation is found in the stages of development of the pollen mother cells in the different locules of the same anther. In an extreme case, the cells of one locule were found in the open spireme stage and those of another locule of the same anther at the end of the homoeotypic division. The cytoplasm is dense and stains darkly, and the walls are extremely thin. A delicate network of chromatin threads occupies all of the nuclear cavity except that part immediately around the nucleolus (fig. 2).

Distinct thickenings can be seen on the threads which make up the network. These thickenings are of the same composition as the other parts of the thread, and are noticeable only on account of their greater size and density. No evidence of "linin" has been observed.

No particular part of a thread can be followed very far through the nucleus with any degree of certainty, because the threads are greatly entangled and run in all directions. It is impossible to determine how many threads make up the network, but free ends have been seen only in those nuclei which were cut in sectioning the material. From observations made at this stage and later, it seems most probable that the chromatin network consists of two univalent threads. The threads show no indication of double nature at this time.

Occasionally two or more nucleoli are found, but usually only one is

present. It most frequently has a lateral position in the nucleus and very small droplets are often seen on its surface. The nuclear membrane is quite distinct. The chromatin threads soon begin to draw away from the nuclear membrane and become more or less parallel to one another (fig. 3). This is the zygotene stage described by Grégoire (17), and is particularly significant because at this time the chromatin thread is becoming bivalent. The bivalent condition is due to the close proximity of the parallel threads. The two members of a pair sometimes approach each other at all regions simultaneously, but more frequently the pairing begins at one point and extends along the threads (fig. 4, *a*, *b*, *c*). This manner of doubling of the chromatin thread was shown by Winiwarter (30) in certain mammals in 1900, by Schreiner (25) in the polychaete *Tomopteris* in 1904, and later by a number of other investigators in still other forms. Very recently Beal (3) has shown that this type of synapsis occurs in cotton. In alfalfa, the members of the pairing threads appear to be completely united in some parts of the nucleus while still quite separate in others.

While the pairing is in progress the entire network contracts and often draws to one side of the nucleus. Sometimes it takes a position on the side of the nucleus but more frequently near the center (figs. 5-7). At the beginning of the contraction period the pairing is incomplete, but pairing and contracting continue *pari passu* until the chromatin material forms a thready ball (fig. 6). The threads therefore undergo the processes of pairing and contracting at the same time, and the pairing is not consummated until mid-synizesis. The process of pairing can be plainly seen in those portions of the threads last drawn into the synizetic knot (fig. 5).

The nuclear cavity now enters upon a period of enlargement which continues until after synizesis. Although much variation occurs in the size of the nuclei, the largest ones found in stages later than synizesis have a diameter of almost twice that of cells in the earliest prophase stages.

The nucleolus becomes somewhat flattened and often assumes a position near the middle of the nuclear cavity. This flattening does not reach its culmination, however, until a considerably later stage (fig. 9). In material which is stained faintly with haematoxylin the nucleolus sometimes appears vacuolate.

In passing out of synizesis the thread is plainly bivalent, and the knots of chromatin have disappeared, leaving the thread more uniform. It has thickened somewhat and often forms a large loop as it begins its passage out of synizesis (fig. 7). A network occupying most of the enlarged nuclear cavity is now formed by the bivalent thread (fig. 8), and the chromatin material loses some of its affinity for stains. The cytoplasm, except around the periphery of the nucleus, becomes less dense and stains lighter than in the preceding stages (fig. 8). The nucleus is now extremely large in comparison with the size of the entire protoplast.

In the cytoplasm just outside the nucleus beaded threads can be followed for considerable distances, and it is probable that these are the anlagen of spindle fibers. No suggestion of the intra-nuclear origin of spindle fibers was found. Allen (2) postulates that nuclear spindle formation would occur in connection with an unusual size of the nucleus, and cytoplasmic along with a greater proportionate supply of extra-nuclear kinoplasm. In *Medicago sativa*, however, there is evidence of cytoplasmic spindle formation in cells having relatively large nuclei. During synizesis the protoplast separates from the original cell wall at the angles and begins to round up (fig. 8). The space left by the withdrawal of the protoplast becomes filled with a homogeneous substance which gives the reaction for callose when tested with resorcin blue or aniline blue.

The homologous members of the bivalent thread become loosely twisted about each other and occasionally some parts of the thread can be seen radiating out from the center to the nuclear membrane, showing some resemblance to the so-called "second contraction." Figures showing such an arrangement have no significance; in fact, the chromatin thread does not usually pass through such a stage, as was claimed by Farmer and Moore (10, 11), Montgomery (22, 23), and other students. They are very difficult to find and can be easily accounted for as occurring by chance. Not a single cell has been observed in which a very large proportion of the thread was arranged in this manner.

The double thread remains slightly twisted and soon shows signs of becoming segmented. The process of segmentation appears to occupy a considerable period of time. The initiation of the process is shown by the appearance of delicate regions in the chromatin thread (fig. 9). This condition is followed by the formation of dark-staining pairs of lumps which alternate in the thread with the above mentioned thin, light-staining regions (fig. 10). These pairs of dark-staining bodies are seen to be about sixteen in number, which is comparable to the haploid number of chromosomes definitely determined in later stages.

The chromosomes then take the position in the nucleus characteristic of diakinesis. While in this stage, the members of a pair are loosely associated (fig. 11) and their bivalent nature is easily determined. In late diakinesis or early metaphase they come more closely into contact but the members of a pair can usually be distinguished at this stage also (fig. 12).

A multipolar spindle was not observed, although a transitional stage between diakinesis and metaphase was found and is shown in figure 12. In this cell the chromosomes are arranged in a triangular plane somewhat like the arrangement of chromosomes in a tripolar stage. A spindle has never been observed, however, earlier than the metaphase. The limits of the nucleus disappear, and a bipolar spindle is formed in the central region of the cell. The spindle is apparently made up of definite fibers which often unite laterally into tufts. Some are attached to the chromosomes

and others pass between the chromosomes from pole to pole. The members of the bivalent chromosomes separate and pass to the poles (fig. 13).

During the anaphase, the chromosomes are unmistakably univalent, showing that reduction has occurred. The univalent chromosomes show no sign of splitting at this time. After the chromosomes have reached the poles the spindle fibers shorten and the equatorial region of the cell appears darker (Pl. VII, fig. 15) suggesting a movement of material from the ends of the spindle to the equator. This dark staining equatorial region represents the last trace of a spindle. In material fixed with Benda's fluid according to directions followed by Devise (9), this region is very pronounced and sometimes appears slightly vacuolate. After making a careful study of the effects of various killing solutions and comparing fixed with living material, the conclusion was drawn that the vacuolation and extreme condensation of cytoplasm at the equator resulting by the use of this fixative were artifacts. No cell plate is formed.

The Homoeotypic Nuclear Division

The univalent chromosomes, after reaching the poles, first fuse into a mass, but this soon loosens up and the chromatin of which it is composed becomes scattered. Small lumps of chromatin often lie along the nuclear membrane, and nucleoli appear in the nuclear cavity. Thus the daughter nuclei undergo a complete reorganization.

Often as many as six or seven nucleoli occur in a single nucleus and as a result the chromatin becomes very scanty (fig. 14). At the earliest stage in which this condition appears, all of the nucleoli are of approximately the same size. Later, however, there is a marked decrease in the size of all of the nucleoli except one, which does not appreciably change in size (fig. 16). This behavior of chromatin and nucleoli, although not studied in detail, indicates a genetic relationship between these structures as Wager (28) has suggested.

The formation of chromosomes from the chromatin of the nucleus proceeds rapidly. New spindles are formed, the chromosomes arrange themselves on the spindle, and the halves pass to the poles in the usual way. The two homoeotypic spindles are usually at right angles to each other (fig. 17), but occasionally they are almost parallel (fig. 18) or else intermediate. When they arise parallel to each other, or when they are intermediate, their orientation apparently changes and when the division is complete, a tetrahedral arrangement of the microspores is the result. Not a single tetrad has been observed with a bilateral arrangement of microspores. After the chromosomes reach the poles, they again go into the resting condition and remain quiet for a relatively long time.

Chromosome Numbers

The haploid number of chromosomes in both varieties studied is sixteen (figs. 12, 13), and the diploid number thirty-two (fig. 26). Counts in

pollen mother cells have been made not only in diakinesis and in the anaphase but also from the polar view of the metaphase. Some differences between the chromosomes have been observed, but these differences do not seem to be consistent. They may be due to the different forms taken by the chromosomes at various stages. No attempt has been made to identify the various members of the chromosome complex.

Division of the Cytoplasm and Formation of the Cell Wall

As the chromosomes reach the poles of the homoeotypic spindles, four other spindles arise out of the cytoplasm, making six in the cell. These spindles are arranged in such a way that each nucleus is connected with each of the others (fig. 19).

The spindles disappear in much the same manner as at the end of the heterotypic division. By staining with gentian violet the spindle can be easily distinguished from the cytoplasm, although the individual fibers are not often seen. As in the heterotypic division, the last trace of the spindle is seen in the region of the equator (fig. 20) and no cell plate is formed. Further, the cytoplasm in the equatorial region may be caused to condense greatly and become vacuolate by improper fixation. This occurs before the normal division of the cytoplasm takes place and is entirely different from the slight vacuolation which normally occurs later and is active in cytokinesis.

These peculiar artifacts have caused difficulty in studying the division of the cytoplasm because at first they appeared to bear some relation to cytokinesis. In well fixed material, however, incipient cytokinesis such as is shown in figure 21 is found in abundance, and from the amount of invagination of the cell wall, it is clear that this figure represents a stage between figures 19 and 22.

The formation of the wall is directly preceded by a slight vacuolation of the protoplasm which assists in its division. The individual vacuoles of this region are extremely small and are usually difficult to see, but the narrow hyaline area caused by this vacuolated condition is often observed (figs. 22, 23). This area predetermines the plane of cleavage of the microspores.

Cytoplasmic division by vacuoles is not uncommon in lower plants, and has been well proven in a few higher plants. An example of this was reported in *Melilotus alba* by Castetter (8). He found that vacuoles of unusual size develop in rows extending midway between the nuclei and reaching from the periphery to the center of the protoplast. These vacuoles by fusion form larger vacuoles and thus leave only a few strands of cytoplasm connecting the four protoplasmic masses. The remaining strands are finally severed by the ingrowing callose wall. Weinstein (29) has shown similar hyaline regions in corresponding stages of *Phaseolus vulgaris*, but does not discuss them.

Lutman (18) is of the opinion that the vacuolation along the plane of cleavage can best be interpreted as the result of a movement of liquids away from the equatorial region to the daughter nuclei and that this movement of liquids brings about the formation of a vacuolated region, the cell plate, across the equator. He believes that the movement of these liquids is through the spindle fibers which are in reality only long, slender tubes of cytoplasm. He holds that the liquid consists chiefly of karyolymph, which is left at the equatorial region when the chromosomes go to the poles, and must be moved to the daughter nuclei in order to assist in the hydrolization of the chromatin and the linin.

The movement of liquids away from the equatorial region cannot be expected to bring about vacuolation in that region, however. Since vacuoles are generally considered to be filled with liquids, the movement of liquids away from any region should tend to make it less rather than more vacuolate. It is, therefore, more probable that the vacuolation is caused by a movement of liquids into the equatorial region, as a result of which the granular cytoplasm is displaced.

The ridges on the inner surface of the pollen mother cell wall in *Medicago sativa* grow towards the center of the protoplast until they meet, and thus divide the latter into four parts. The partition wall then thickens and a dark-staining middle region can be recognized (fig. 24). This region is most clearly seen when fresh material is used, and can be fairly well differentiated with Delafield's haematoxylin in paraffin sections.

A dark-staining middle region in the partition walls of *Ipomoea* and *Oenothera* was reported by Beer (4, 5) who designates it as the inner lamella and states that it is the first layer formed after the completion of cell division. In an earlier paper the present writer (24) has described a structure in partition walls of the pollen mother cells of *Zea Mays* which strongly resembles the dark-staining middle layer in *Medicago sativa*. In *Zea Mays*, however, the method of wall formation is entirely different. Here the walls are formed by cell plates.

The formation of cell walls by furrowing has long been known to occur in animals and in lower plants; and it was formerly believed that all cell walls of higher plants were formed by cell plates. In 1916, however, Farr (12-15) began the publication of a series of papers which proved that in a number of higher plants partition walls of microspore mother cells were formed by furrowing. As an explanation of this process he postulates in his first paper (12) that the nuclei of the tetrad bear electrical charges of like sign. He shows that certain forces will then be exerted in such a way as to transform the protoplast into a tetrahedron with four equal triangular faces, each parallel to the plane of three of the nuclei. The continued exertion of these forces will cause the center of each triangular face to move towards the fourth nucleus and an invagination of the protoplast by the plasma membrane will be the result.

Other investigators have explained furrowing as being the result of surface tension phenomena. Bütschli (7) and McClendon (19-21) have supported the view that invagination by the cell wall is a result of high surface tension at the equator of the cell. Spek (26-27) has added support to this view by showing that lowering of the surface tension of the opposite poles of oil droplets in water causes an invagination along the sides of the droplet and divides it into two parts.

In *Medicago sativa*, after the formation of the wall is complete, the microspores begin to increase in size, apparently by the digestion of their thick, mucilaginous walls. The original walls of the pollen mother cells disintegrate last.

Development of Pollen to Maturity

The microspores continue to increase in size until they are several times their original diameter. In the younger stages their cytoplasm is very vacuolate and as a consequence the spores are easily shrunken during fixation. The wall of the pollen grain differentiates into intine and exine and has three sulci which make the grain slightly three-lobed. When plasmolysis occurs the walls fold inward along the sulci and the grain becomes more conspicuously lobed. When in a turgid condition, however, it is almost spherical (fig. 25). Three germ pores occur, one near the middle of each sulcus.

Soon after the spore is formed its nucleus divides and gives rise to two daughter nuclei of unequal size. Before dehiscence of the anther the smaller nucleus takes a position in one side of the protoplast and a spindle shaped region of cytoplasm differentiates around it.

DISCUSSION

The chromosome numbers of species of many genera of plants and animals have been observed to range in multiples of a basic number. This has been recently emphasized by Aase and Powers (1) who have summarized the literature and contributed some original data on chromosome numbers. The most recent complete summary of chromosome numbers in angiosperms was published by Gaiser (16) in 1926. Apparently the chromosomes of *Medicago sativa* have not been counted, but some counts have been made in related plants. Bleier (6) reported the haploid number as being seven in *Trifolium campestre*, *T. badium*, *T. glomeratum*, *T. arvense*, and *T. pratense*. He reported eight in *T. hybridum*, *T. fragiferum*, *T. resupinatum*, *T. lappaceum*, *T. incarnatum*, *T. alpestre*, and *T. ochroleucum*, and fourteen in *T. minus* and *T. repens*. Castetter (8) found eight to be the haploid number and sixteen the diploid in both annual and biennial varieties of *Melilotus alba*.

The literature at hand shows quite clearly that cell wall formation in the higher plants may occur by various methods. The opinion is generally

held that the walls are formed in monocotyledons by successive bipartitions and in dicotyledons by simultaneous quadripartition. Farr (14), however, as a result of his work on *Sisyrinchium* and a careful review of the literature has shown that quadripartition by furrowing sometimes occurs in monocotyledons.

That bipartition occurs by cell plates has often been reported, and the present writer (24) has shown the details of this process in *Zea Mays*. The first part of the wall formed takes a darker stain than the younger layer, and may be considered as the middle lamella. It differs from the middle lamella usually observed in somatic cells only in chemical composition. The structure and chemical composition of the partition walls of pollen mother cells formed by cell plates and by furrows, as in *Medicago sativa*, are therefore much alike.

SUMMARY

1. When the pollen mother cell of *Medicago sativa* enters the prophase of the heterotypic division, the chromatin is in the form of a network and occupies the entire nuclear cavity except that part occupied by the nucleolus.

2. The chromatin thread becomes bivalent as it enters synizesis.

3. During synizesis the pairing is consummated, and the thread becomes larger.

4. The bivalent nature of the thread can be seen in all stages after synizesis.

5. The haploid number of chromosomes is 16; the diploid, 32.

6. The daughter nuclei undergo a complete reorganization at the end of the heterotypic division. They often contain several nucleoli, some of which are probably composed of chromatin material.

7. The last traces of the disappearing spindle fibers are seen along the equatorial region. No cell plates are formed in either the heterotypic or the homoeotypic division.

8. Cytoplasmic division occurs by ingrowing ridges on the pollen mother cell wall, accompanied by slight vacuolation.

9. A dark region comparable to a middle lamella can be observed in the partition walls when properly stained.

10. Division of the nucleus of the pollen grain occurs before the pollen is shed. The generative nucleus then becomes surrounded by a lens-shaped region of cytoplasm and takes a position on one side of the pollen grain.

This investigation was initiated in the Plant Morphology Section of the Iowa State College under the direction of Dr. J. N. Martin, to whom the writer is indebted for advice and criticism. The work was carried to completion in the Department of Biology, Agricultural and Mechanical

College of Texas, and grateful acknowledgment is due Dr. O. M. Ball, head of this department, for his encouragement and valuable suggestions.

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EXPLANATION OF PLATES

The figures were drawn with the aid of an Abbe camera lucida under several different combinations of Bausch and Lomb apochromatic objectives and compensating oculars. The approximate magnification of each figure is given.

PLATE VI

- FIG. 1. Longitudinal section of anther at the beginning of the heterotypic division. Locule wall fully formed. $\times 266$.
- FIG. 2. Pollen mother cell as it enters the prophase. $\times 2000$.
- FIG. 3. Nucleus showing zygotene stage. $\times 2000$.
- FIG. 4. *a, b, c.* Portions of zygotene thread. $\times 2000$.
- FIG. 5. Very early synizesis. $\times 2000$.
- FIG. 6. Mid-synizesis. $\times 2000$.
- FIG. 7. Very late synizesis. $\times 2000$.
- FIG. 8. Open spireme. Protoplast spherical. $\times 2000$.
- FIG. 9. Earliest sign of segmentation. Threads slightly twisted about each other. $\times 2000$.
- FIG. 10. Segmentation almost complete. $\times 2000$.
- FIG. 11. Diakinesis. Ten bivalent chromosomes and a fragment are shown. The remainder of sixteen were found on the next section. $\times 2000$.
- FIG. 12. Transition stage between diakinesis and metaphase. Sixteen bivalent chromosomes. $\times 2000$.
- FIG. 13. Polar view during anaphase of heterotypic division. Sixteen univalent chromosomes. $\times 2000$.
- FIG. 14. Daughter nucleus formed at end of heterotypic division. Several nucleoli. Chromatin scanty. $\times 2000$.

PLATE VII

- FIG. 15. End of heterotypic division. Dark equatorial region caused by remains of spindle. $\times 1500$.
- FIG. 16. Slightly later. No remains of spindle seen. $\times 1530$.
- FIG. 17. Homoeotypic metaphase. Spindles at right angles. Details omitted. $\times 1300$.
- FIG. 18. Same. Spindles parallel. $\times 1300$.
- FIG. 19. Protoplast at end of homoeotypic division. Only three nuclei in one plane. The fourth one is below; not shown. $\times 1500$.

FIG. 20. Later stage. Remains of spindles seen along equatorial regions. $\times 1500$.

FIG. 21. Remains of spindle have disappeared. $\times 1150$.

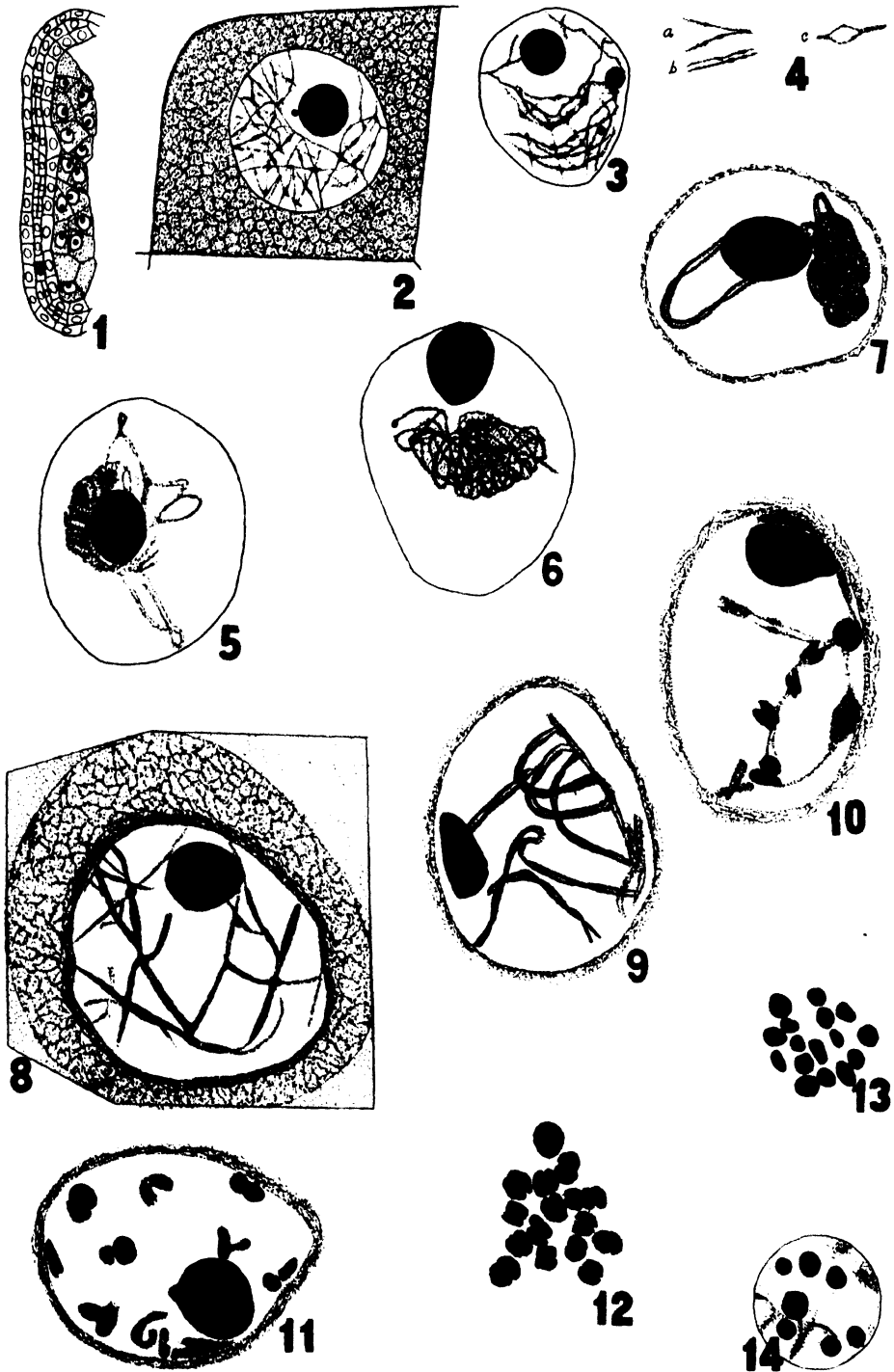
FIG. 22. Slight vacuolation accompanied by invagination of the cytoplasm by the mother cell wall. $\times 1100$.

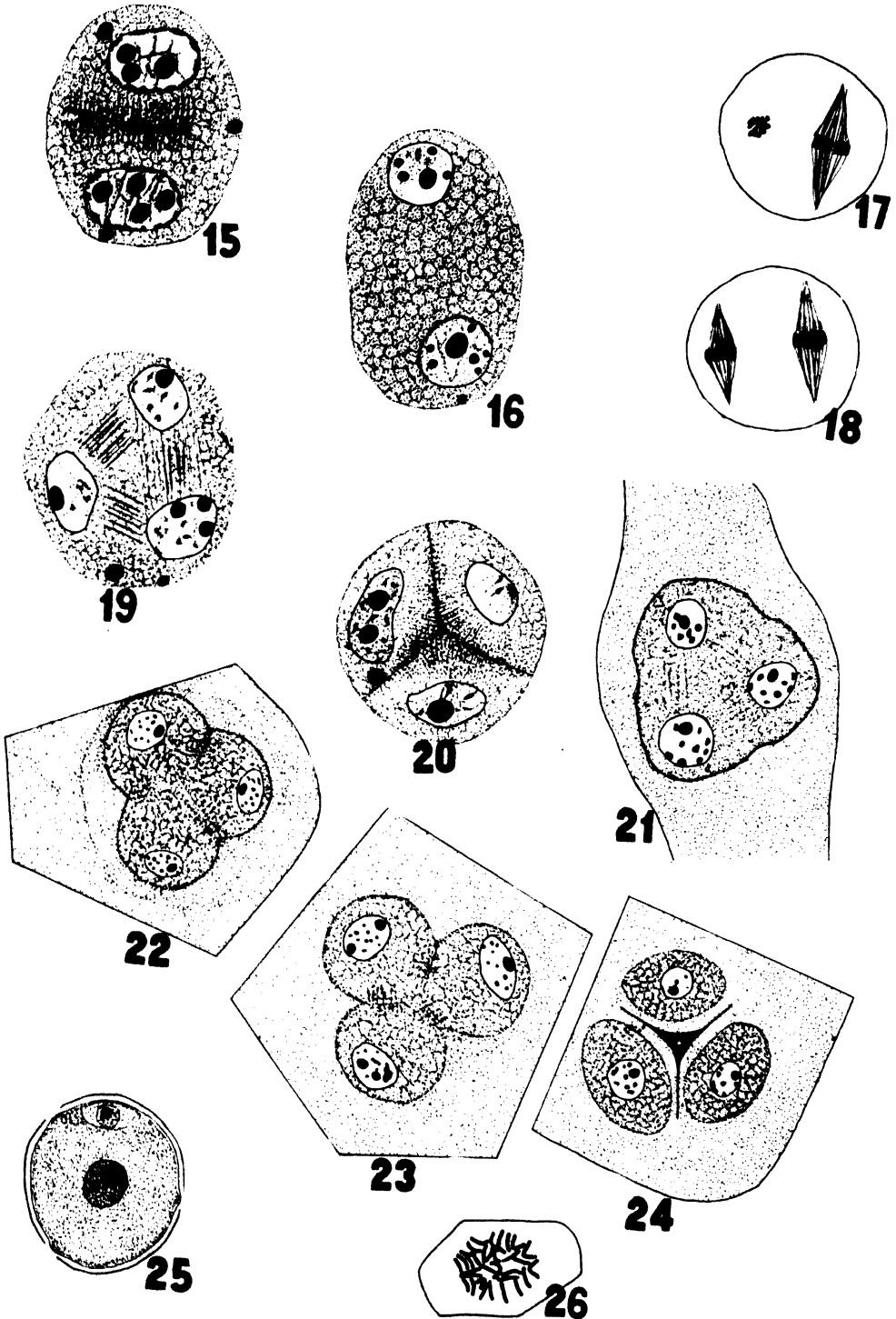
FIG. 23. Later stage. $\times 1330$.

FIG. 24. Tetrad of microspores completely formed. Dark staining middle lamella present. $\times 1100$.

FIG. 25. Mature pollen grain. $\times 750$.

FIG. 26. Cell of root tip. Thirty-two chromosomes. $\times 1000$.





REEVES: ALFALFA MICROSPOROGENESIS

SPECIES CROSSES IN THE GENUS *CUCURBITA*

EDWARD F. CASTETTER

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Of the ten species comprising the genus *Cucurbita* only three, *C. Pepo* L., *C. maxima* Duchesne, and *C. moschata* Duchesne are annual, and these are the only species cultivated in the United States. Obscurity has surrounded their origin and this has given rise to much discussion regarding their nativity. Some convincing evidence regarding the North American origin of *C. Pepo* has been furnished by Small (21), who on collecting excursions to Lake Okeechobee, Florida, discovered a "gourd" growing on the southern shores of the lake, which he later found to be very similar to our present cultivated pumpkin. Small states that the foliage, flowers, and seeds of this wild pumpkin are indistinguishable from our economic forms of today, but that the fruit is much smaller, ranging in size from a baseball to that of a croquet ball. The color is pale yellow, and sometimes variegated with green markings. From a "memoir" of Hernando de Escalante Fontaneda, written in Spain about 1575, Small quotes a description of what is no doubt the same general region in which his own excursions were made. One of the regions is designated by Hernando de Escalante Fontaneda as Tocola-a-chile, which when translated means "Gourd place bringing forth," or "Country where gourds are produced." Small (21) believes that the wild pumpkin which he found near Lake Okeechobee is the same as the "gourd" mentioned in the old Spanish record of exploration in Florida, and that the Lake Okeechobee region, including the unexplored hammocks of Lake Istokpoga, to which this wild pumpkin is at present restricted, represents the original home of the pumpkin. Small (21) also observes that the Seminole pumpkin, which pioneer white men found among the Seminole Indians in the region now known as Florida, still grown by the Seminole Indians of today, is a cultivated form of the wild type described above. "The stem and flowers are identical with those of our various cultivated pumpkins. The fruits are larger than in the wild plant—and they vary from spheroidal, often much depressed, through pyriform to those with a short stout neck." It seems quite likely that the pumpkins found among Indian tribes by the early settlers, as well as among some tribes of the present day, have come up through the Seminole pumpkin from the original wild form of the Lake Okeechobee region. This wild pumpkin described by Small (21) belongs to *Cucurbita Pepo* and may be considered the prototype of this species.

Evidence regarding the origin of the other two species is less tangible. Wittmack (24) believes pumpkins and squashes native to America, as he found seeds of *Cucurbita maxima* in old Peruvian tombs; also this same species was observed by DeSoto in Florida in 1542 and known to be grown by North American tribes as far north as the St. Lawrence River, according to Pickering (20). Belief that this species is native to the old world, however, is supported by de Candolle (5), while Naudin (18) believed that all three of the cultivated species grown in this country originated in the old world.

In the literature dealing with origin of the members of the genus *Cucurbita* the terms "squash" and "pumpkin" are applied with no consideration for botanical relationships; nor could they be, as neither of these names has heretofore been assigned to a particular species. Hence, it is impossible to infer the species involved when either of the terms is used.

The origin of the word "squash" has been attributed to the American Indian word "wata hti" (real squash)—"wata miha sned" (long squash), etc. One of the southern clans of the Hopi Indians was called the "Patun," or squash, family, according to Fewkes (8), who also found squash seed in some of their mortuary bowls. Other convincing evidence of the antiquity of the squash is allusion to it in some of the very oldest religious songs of the Pima tribe, as reported by Gilmore (9), who reminds us that "religious expression is one of the most conservative elements and does not take on anything new." In his history on the Cheyenne Indians, Grinnell (11) states the squash has been cultivated by them from earliest time. In missionary work among the Indians Dr. Elmer E. Higley, of Ames, Iowa, learned that the squash blossom signified fertility and was regarded as sacred by the Hopi Indians; also that it was emblematic among the Navajo as is evidenced in its use by their silversmiths.

The matter has been well summed up by Gray and Trumbull (10), who state: "We find abundant evidence, especially as respects North America . . . that (1) in various parts of the country, remote from each other, the cultivation of one or more species of cucurbits by the Indians was established before those places are known to have been visited by Europeans; (2) these species or varieties were novel to Europeans, and were regarded by botanists of the sixteenth and seventeenth centuries, as well as by the voyageurs and first colonists, as natives or denizens of the region in which they were found; and (3) they became known only under American names; one of these names (squash) becoming, in popular use, generic, and two others (macock and cushaw) surviving as names of varieties into the present century."

An additional interesting comment has been offered by Sturtevant (22): "If we consider the stability of types, and the record of variations that appear in cultivated plants, and the additional fact that so far as determined the originals of cultivated types have their prototype in nature, and are

not the product of culture, it seems reasonable to suppose that the record of the appearance of types will throw light upon the country of their origin. From this standpoint, we may hence conclude that, as the present types (pumpkins) have all been recorded in the Old World since the fifteenth century, and were not recorded before the fourteenth and succeeding centuries, there must be a connection between the fact of the discovery of America, and the fact of the appearance of pumpkins and squashes in Europe."

DESCRIPTION OF SPECIES

During the past six years all the commonly cultivated varieties of the three annual species of *Cucurbita* have been grown and observed with a view to making more complete species descriptions than those heretofore available, which have been based on observation of only a small number of varieties. Each variety has been grown and studied at least two different seasons, and each of the following descriptions is a composite of all varieties comprising that species.

***Cucurbita Pepo* L.**

Plants with running or bushy, five-sided, usually prickly stems, which in the running varieties have distinct ridges and grooves. Leaf stalks and blades spiny, the blades three to seven lobed with distinct sinuses between the lobes. Both staminate and pistillate flower stalks obtusely five-sided. Corolla orange-yellow, the tube flaring and lobes pointed. Calyx tube of staminate flower bulbous, slightly constricted just below the sepals; that of pistillate flower short and disc like. Sepals distinctly tapered, fleshier than in *C. maxima*, and those of the pistillate flower reduced in size. In contrast with *C. maxima*, the tip of the corolla remains pointed up to the time of the opening of the flower. Fruit stalk very hard at maturity, five-sided, distinctly grooved; often slightly enlarged or flaring at attachment to fruit. Shell of fruit hard at maturity, not yielding readily to the thumbnail. Seeds tan colored, with a horizontal or rounded scar; margin identical in color and texture with body of seed.

***Cucurbita maxima* Duch.**

Vines strongly running, stems cylindrical or nearly so, but not grooved. Stem, leaf blades, and leaf stalks differ from those of *C. Pepo*, being rough hairy rather than spiny. Leaf blades somewhat kidney shaped, without distinct sinuses between the rounded lobes. Staminate flower stalk cylindrical. Corolla tube of both flowers nearly cylindrical, the lobes rounded and reflexed. Calyx tube of staminate flower roughly cone shaped, that of pistillate flower disc like; sepals of both flowers linear. On the evening previous to the opening of the flower, the tip of the corolla spreads somewhat. Fruit stalk cylindrical in outline, fleshy, remaining soft at maturity. Shell hard in some varieties, soft in others. The color of seeds is usually

white, but may be brown or bronze. This species is characterized by a slanting seed scar.

***Cucurbita moschata* Duch.**

This species is much more variable than either *C. Pepo* or *C. maxima*, it being difficult to ascribe definite characteristics which cover all varieties. The plants have running stems, which in most cases are five-sided; in a few forms, however, they are rather cylindrical with narrow ridges and grooves. Leaves and stems usually soft hairy, or rarely rough hairy; leaf blades with three to five lobes, and with very few exceptions characterized by white spots at intersections of veins. These spots, however, differ in appearance from those which occasionally occur on the leaves of *C. Pepo*. Flowers coarser and firmer than in other species. Corolla lemon-yellow, the tube somewhat flaring, with lobes intermediate in shape between the pointed of *C. Pepo* and the rounded of *C. maxima*. Peduncles more or less five-sided, with or without grooves. With few exceptions this species has noticeably long stamens. Calyx tube of staminate flower quite variable in shape, that of pistillate disc-like, both often five-sided and flanged at top; sepals long or short, rather flat, with or without leaf-like terminations. As in *C. Pepo*, and in contrast to *C. maxima*, the flowers on the evening previous to opening are pointed at the tip. Shell of fruit (with a single exception) hard, thin or thick. The fruit stalk is typically five-sided, noticeably grooved and distinctly flaring at attachment to fruit. However, in a few varieties these characters are somewhat modified, that is, roughly cylindrical in outline, indifferently grooved, and neither flaring nor noticeably enlarged at attachment to fruit. Fruit stalks are hard or medium hard, never soft as in *C. maxima*. Seed scar slanting, horizontal or rounded. The seeds of this species range from a grayish white to tan, the distinct margin thickened, deeper in color, and different in texture from the body of the seed.¹

These descriptions of *Cucurbita Pepo*, *C. maxima*, and *C. moschata* embrace and supplement, in modern and simple terminology, the original descriptions by Linnaeus and Duchesne. It will be seen the characters of *C. Pepo* and *C. maxima* are clean cut and definite, whereas those of *C. moschata* are difficult of demarcation. Also, this species has some characters similar to those of *C. Pepo*, and others not unlike those of *C. maxima*.

Duchesne in his original description of *Cucurbita moschata* states: "This species, very difficult to delimit, is comprised of several varieties too little observed to determine well." Also: "Lamarck did not find sufficient differences to consider it a distinct species." The present indefinite and inconstant characters of *C. moschata*, together with the uncertainty of Duchesne and Lamarck in recognizing it as a separate species, suggest the possibility of hybrid origin.

¹ The distinguishing characters of the above species of *Cucurbita* have been figured in an earlier paper by Castetter and Erwin (4), and hence are omitted here.

CYTOLOGICAL INVESTIGATIONS

In an earlier paper the writer (3) reported on the cytological behavior in *Cucurbita maxima*. The number of chromosomes in a pure line of Hubbard squash (*C. maxima*) was found to be haploid 20, diploid 40. Recently the chromosome numbers in inbred lines of Connecticut Field pumpkin (*C. Pepo*), designated line No. 175, and of Large Cheese pumpkin (*C. moschata*) line No. 5, were investigated. In *C. Pepo* the haploid number was found to be 20, the diploid 40; in *C. moschata*, however, the haploid was 24, diploid 48. No difference in size or shape of chromosomes was found among the three species, nor was it possible to find any other cytological distinction between these species with respect to microsporogenesis and nuclear division in the root tip. Megasporogenesis has not been investigated.

The above cytological results, however, are at variance with those of Lundegardh (17), and Kozhukhow (14). Lundegardh reports a haploid number of 12 in *C. Pepo*, although he merely counted the lumps of chromatin in the nucleus. Kozhukhow investigated two forms of *C. Pepo*; for one he claims a minimum diploid number of 42 and thinks there may be as many as 44 or 46. For the other form he finds the number to be from 2 to 4 less than in the first, although his plate shows 40 diploid. He finds that in *C. maxima* and *C. moschata* the number is not less than 44 and probably not more than 48.

HISTORY OF SPECIES CROSSES IN CUCURBITA

As early as 1854 Naudin (18) attempted to intercross various species of *Cucurbita*, viz: *C. maxima*, *C. Pepo*, *C. moschata*, *C. melanosperma*, and *C. perennis*. The result of a comparatively small number of controlled pollinations was a few fruits, none of which contained fertile seeds.

More than thirty years later a similar line of investigation was undertaken by Bailey (1, 2). He worked for ten consecutive years cross-pollinating varieties, species, and genera of the Cucurbitaceae. As a result of numerous efforts with the three cultivated species of *Cucurbita*, seven fruits were secured: two by pollinating *C. Pepo* \times *C. maxima*, one by *C. moschata* \times *C. maxima*, one by *C. maxima* \times *C. Pepo*, and three by *C. Pepo* \times *C. moschata*. However, fertile seeds were found only in the three fruits obtained from *C. Pepo* \times *C. moschata*, and eighty-eight F_1 plants were grown from the seeds in the two fruits resulting from a cross of Connecticut Field pumpkin and Japanese Crookneck. (No mention is made of the seeds from the third fruit, Gourd \times Japanese Field pumpkin.) A number of F_2 plants were also grown and one fruit secured, but Bailey does not state whether this fruit contained fertile seeds. Somewhat at variance with the findings of Bailey are those of Pammel (19) who concluded that it is impossible to obtain hybrids between the different species of *Cucurbita*.

Additional data have been furnished by Drude (6, 7), whose researches extended over a period of twenty-five years. Only three fruits were produced and these by the pollination of *C. Pepo* (white apple) with *C. ficifolia* (*C. melanosperma*). Two of these fruits were entirely sterile, the third contained one fertile seed. This hybrid was in turn successfully crossed with a Fordhook (*C. Pepo*). Drude's attempts between 1901 and 1905 to repeat this cross between *C. Pepo* and *C. ficifolia* failed, as did all attempts to cross other species of the genus during his twenty-five years of experimentation.

The work of the Hagedoorns (12, 13) is of considerable interest in that they not only secured several interspecific hybrids (*C. maxima* \times *C. Pepo*), but also apparently found that some of the F_1 hybrid plants set fruit and produced viable seeds from unpollinated female buds. Only three F_1 interspecific and two F_1 intervarietal hybrids developed parthenogenetic seeds. The F_2 and F_3 generations in each case were grown for the purpose of ascertaining whether true parthenogenesis or merely apogamy was indicated. By this genetic method it was found that in at least four of the five cases the progenies gave strong evidence of parthenogenesis. That parthenogenesis in *Cucurbita* is closely associated with hybridity finds support in the fact that 106 female flowers on 18 varieties, carefully protected from pollination, failed to produce a single seed. It is worthy of note that the three fruits resulting from the pollination of *C. maxima* (Turkenbund) \times *C. Pepo* developed on a single Turkenbund plant. Their efforts to intercross species other than *C. maxima* \times *C. Pepo* were unsuccessful.

In discussing the investigations carried on by the Hagedoorns, Lotsy (15, 16) thinks the possibility of crossing *C. maxima* with *C. Pepo* has not been proved. Although he made unsuccessful attempts to cross these species himself, he feels his work has not been sufficiently extensive to conclude definitely that crosses cannot be made between *C. maxima* and *C. Pepo*. He is convinced, however, from his own investigations that neither *C. Pepo* nor *C. maxima* can be crossed with *C. melanosperma*. Lotsy's results do not confirm those of the Hagedoorns regarding the occurrence of parthenogenesis in unpollinated F_1 hybrids.

The most recent work with a view to crossing the species of *Cucurbita* is that of Vavilov (23), who made reciprocal pollinations among four species, *C. Pepo*, *C. maxima*, *C. moschata*, and *C. melanosperma*. Not only was he unsuccessful in obtaining hybrid seed, but in not a single case was fruit secured as a result of his pollinations. He, too, was unable to confirm the phenomenon of parthenogenesis as reported by the Hagedoorns. Vavilov cites the work of Miss Koslov (unpublished) at the Turkestan Agricultural Experiment Station, who secured four hybrid seeds by pollinating *C. maxima* with *C. moschata*. The F_1 fruits were entirely without fertile seed, as Vavilov personally observed in December, 1924. His conclusion is:

"Species of Cucurbitaceae are so different that to get fertile hybrids among them, and especially among different species of *Cucurbita*, is impossible."

Thus as a result of interspecific hybridization, a considerable number of fertile F_1 hybrids of *C. Pepo* \times *C. moschata* were obtained by Bailey; one F_1 hybrid plant of *C. Pepo* \times *C. ficifolia* (*C. melanosperma*), by Drude; a number of F_1 hybrids of *C. maxima* \times *C. Pepo* by the Hagedoorns, and four sterile hybrids of *C. maxima* \times *C. moschata*, by Miss Koslov (unpublished).

RESULTS OF CROSS POLLINATIONS BETWEEN SPECIES OF CUCURBITA

In the summer of 1922 the writer began an investigation with a view to determining the possibility and extent of species crosses in the genus *Cucurbita*. The earlier work consisted only of pollinations between three species of the genus, viz: *C. maxima*, *C. Pepo*, and *C. moschata*, to determine the limits of crossing, but later included a study of the hybrids resulting therefrom. Plants were all grown on the grounds or in the greenhouse of the Iowa Agricultural Experiment Station.

The investigations were carried on with two general types of seeds: those coming from lines inbred for a number of generations, and those obtained from numerous commercial seed houses throughout the United States. In all cases numbers have been used to designate the inbred lines, while varietal names have been employed for commercial forms.

The sources of the inbred lines are:

- No. 175. Connecticut Field pumpkin (*C. Pepo*) developed by the writer by inbreeding.
- Nos. 48a, b, c. Patty Pan, or Scallop, pumpkin (*C. Pepo*), from E. W. Sinnott.
- No. 270. Hubbard squash (*C. maxima*) from John W. Bushnell, then of the Minnesota Agricultural Experiment Station.
- No. 5. Large Cheese, or Kentucky Field, pumpkin (*C. moschata*), developed by the writer through inbreeding.

As *Cucurbita* is monocious and has very large flowers it was easy to prevent contamination by foreign pollen. The method used was to isolate both staminate and pistillate flower buds by tying the corolla with a heavy cord on the evening previous to opening. On the following morning the cord was removed from each flower and the stamens from the desired variety rubbed over the stigma of the pistillate flower, which was again tied with the cord, properly tagged and labelled—the various types of pollinations being designated by tags of different colors attached to the fruit stalk. Especial care was exercised in tying the buds and flowers to preclude the possibility of pollination by insects; also flowers torn in handling, or those concerning which there was any doubt as to complete isolation, were discarded. Immediately before being removed from the vines all fruits were tagged a second time to prevent confusion of identity in handling.

Fruits were allowed to remain unopened for a month or more after being removed from the field or greenhouse, in order to allow seeds to mature fully.

A summary of investigations carried on in both field and greenhouse over a period of seven years is given in condensed tabular form according to species.

C. Pepo × *C. maxima*

| Parents | Number of Pollinations | Fruits | Fertile Seeds |
|--------------------------------|------------------------|--------|---------------|
| Connecticut Field × Hubbard | 93 | 10 | 0 |
| 175 × 270 | 51 | 4 | 0 |
| 175 × 20 | 70 | 4 | 0 |
| Connecticut Field × Marblehead | 152 | 12 | 0 |
| Connecticut Field × Victor | 120 | 6 | 2 |
| 48a × 20 | 54 | 17 | 0 |
| 48b × 20 | 74 | 8 | 0 |
| 48c × 20 | 53 | 17 | 10 |
| Total | 667 | 78 | 12 |

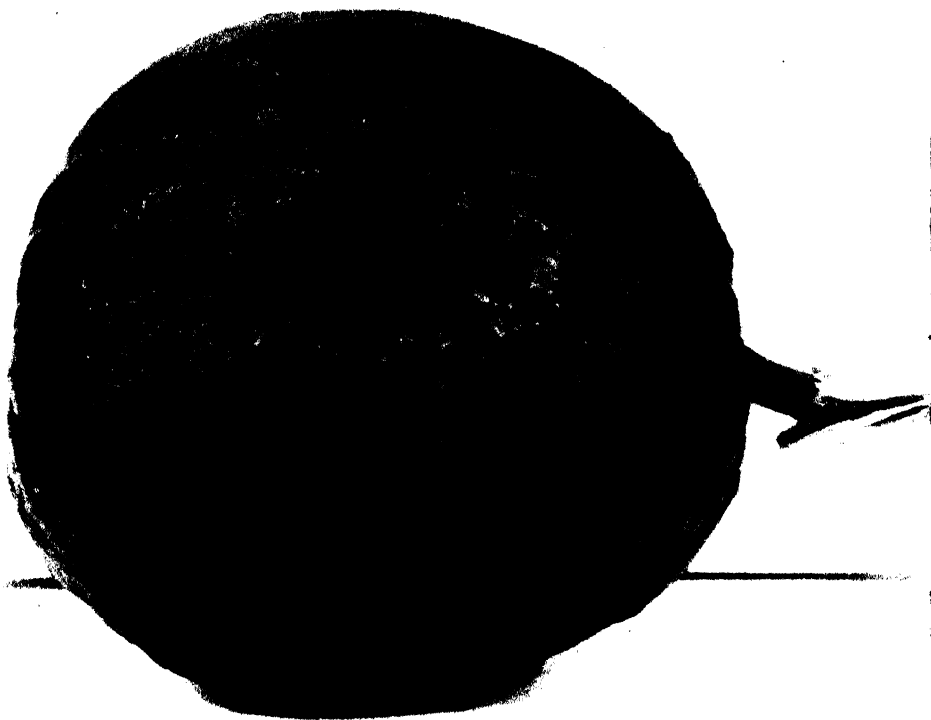
From the above table it will be seen that a number of parthenocarpic fruits were secured. In neither successful cross, however, were the fertile seeds all found in a single fruit. Of the two obtained from Connecticut Field × Victor, one seed was found in each of two fruits. The 10 seeds resulting from the 48c × 20 cross were distributed among four fruits. These 12 hybrid seeds appeared in fruits grown in the field during the summer of 1927. The two seeds from Connecticut Field × Victor gave rise to plants which died before reaching a stage where description was of value.

C. maxima × *C. Pepo*

| Parents | Number of Pollinations | Fruits | Fertile Seeds |
|--|------------------------|--------|---------------|
| Hubbard × Connecticut Field | 176 | 22 | 0 |
| 270 × 175 | 285 | 13 | 0 |
| 20 × 175 | 434 | 38 | 11 |
| Marblehead × Connecticut Field | 142 | 14 | 0 |
| Victor × Connecticut Field | 38 | 7 | 0 |
| Delicious × Connecticut Field | 57 | 5 | 0 |
| 20 unpollinated (flowers tied without pollinating) | 43 | 0 | 0 |
| Total | 1175 | 99 | 11 |

The F_1 of 48c × 20 is designated as No. 55, and the eight plants of this first hybrid generation were very similar to the pistillate parent in all respects excepting the running habit, a character inherited from the staminate parent. A large number of F_2 plants were grown, but as it is apart from the purpose of this paper to present any genetic analysis of

the hybrids no description of this F_2 generation will be given, except to say that the segregation in shape and color of fruit and trailing habit of vine growth clearly showed that undoubtedly a cross between the two species had been made. No effort was made to ascertain the viability of the F_2 seeds.



TEXT FIG. 1. The F_1 of No. 20 (inbred line of Hubbard squash) \times No. 175 (inbred line of Connecticut Field pumpkin), designated as No. 100.

These eleven hybrid seeds from 20×175 appeared as follows. In the field: 1924, 4 seeds in 1 fruit; 1925, 3 seeds in 2 fruits; 1926, 2 seeds in 2 fruits. In the greenhouse: 1924, 2 seeds in 2 fruits. The resulting F_1 hybrid plants were all grown in the greenhouse.

No. 20, an inbred line of Hubbard squash and the pistillate parent of the hybrids, is characterized as follows: rough hairy stems, leaf blades and

leaf stalks; rather kidney-shaped leaves with rounded lobes and indistinct sinuses between the lobes; fruit stalk cylindrical and spongy; shape of the fruit nearly spherical, but pointed at blossom end; surface bumpy; color glossy dark green; shell hard at maturity; size 9×6 inches; weight about 6 pounds.

No. 175, an inbred line of Connecticut Field pumpkin—the staminate parent of the hybrids—has spiny stems, leaf blades and leaf stalks; leaves strongly lobed, with deep sinuses between the lobes; fruit stalk hard at maturity, five-sided, distinctly grooved, not noticeably enlarged at attachment to fruit; fruit round, flattened at both ends; surface smooth; color orange yellow; shell thin and hard; size about 16×10 inches; weight about 20 pounds.

From the eleven F_1 hybrid seeds of 20×175 only four plants grew to maturity, seven of the seeds giving rise to feeble plants which died when from four to six inches tall. These four plants were the progenies of two 20×175 fruits, each fruit from a different plant; three of the seeds coming from one fruit and the fourth seed from another. The single hybrid plant from the first fruit has been designated as No. 100 (text fig. 1), and the three hybrid plants from the second fruit as No. 101.

Three cuttings were made from No. 100 and the three resulting plants were grown in the greenhouse. No. 100 is characterized by prickly stems, leaf blades, and leaf stalks, with leaf blades distinctly lobed, and with deep sinuses very similar to those of the staminate parent, No. 175. These plants bore very few flowers, either staminate or pistillate. All the pollen was abortive, and examination with the microscope showed it to be badly shriveled. Although self pollination of No. 100 resulted in no fruits, several fruits were secured by back-crossing, as will be seen from the following table. These fruits were nearly spherical in shape, varied greatly in size, had hard, thick, warty shells and in color were dark green with a faint gray mottling. The seed coats and fruit stalk closely resembled those of *C. Pepo* (No. 175). None of these fruits contained any fertile seeds.

*Pollination of F_1 Hybrids of *C. maxima* \times *C. Pepo**

| Parents | Number of Pollinations | Fruits | Fertile Seeds |
|---|------------------------|--------|---------------|
| 100 selfed | 6 | 0 | 0 |
| 100 open pollinated in field | | 3 | 0 |
| 100 unpollinated (flowers tied without pollinating) | 12 | 0 | 0 |
| 100×175 | 6 | 2 | 0 |
| 100×20 | 3 | 1 | 0 |
| 20×100 | 5 | 0 | 0 |
| 101 selfed | 9 | 0 | 0 |

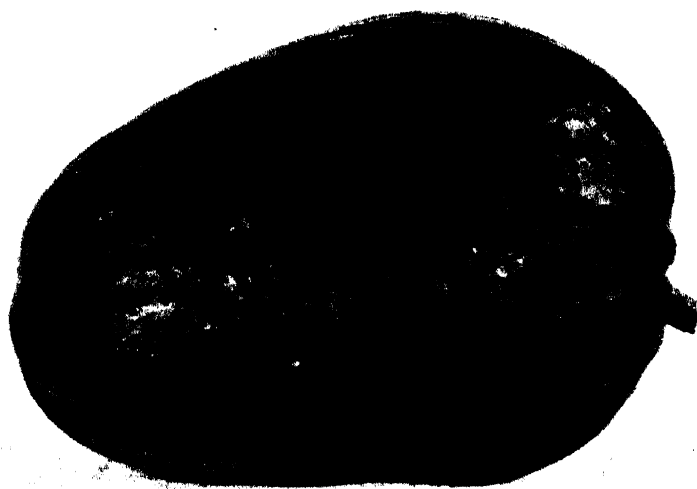
The above description of No. 100 will also serve for No. 101, with the exceptions that the terminal lobe of the leaf of No. 101 was much shorter

than that of No. 100, and the fruit of No. 101 was light green, decidedly mottled with gray and yellow, as opposed to the dark green and faint gray mottling of No. 100. The three No. 101 plants were very similar to each other in all respects.

The small number of pollinations recorded for this first hybrid generation is due to the few flowers formed on these plants. The appearance of the staminate flowers and the pollen, as well as the behavior of these hybrids, indicate that the F_1 is totally sterile.

C. Pepo \times *C. moschata*

| Parents | Number of Pollinations | Fruits | Fertile Seeds |
|---|------------------------|--------|---------------|
| 175 \times 5 | 134 | 14 | 57 |
| Connecticut Field \times Striped Cushaw | 24 | 1 | Many |



236538

125

TEXT FIG. 2. Hybrid No. 125, the first generation resulting from No. 175 (inbred line of Connecticut Field pumpkin) \times No. 5 (inbred line of Large Cheese pumpkin).

The pistillate parent in the cross of 175 \times 5 has been described earlier in the paper. No. 5, the staminate parent, has soft hairy stems and leaves;

leaf blades weakly lobed without notches between the lobes, and with silvery spots at intersections of veins; fruit round, flattened at both ends, giving the appearance of a cheesebox; shell thin, but hard and smooth; color creamy buff; fruit stalk hard, five-sided, deeply grooved and distinctly flaring at attachment to fruit.

The F_1 plants of 175×5 (designated as No. 125, text fig. 2) were grown in both greenhouse and field. The shape of the leaf and the degree of prickliness of leaf and stem were intermediate between those of the parents. The fruit shape was short oblong, and the color a network of green over a light orange background. The network, incompletely covering the background, gave to the fruit the appearance of being splotted. These fruit characteristics are difficult to understand when we consider the shape and color of both parents. The shell of the fruits was hard, smooth or slightly warted, and the fruit stalk flaring at its attachment as in the staminate parent. Self-pollinating No. 125 shows this F_1 generation to be very fertile, as many viable seeds were secured out of the 12 fruits resulting from 19 pollinations.

From these seeds a large number of F_2 plants were grown. Since it is not primarily the purpose of the present paper to report the inheritance of characters in these crosses, no details will be given on the F_2 generation except that it was somewhat fertile, for out of 62 self pollinations of the F_2 (designated as No. 127) 19 fruits with many viable seeds were secured. The F_3 was also grown and found to be quite fertile.

The F_1 generation of Connecticut Field \times Striped Cushaw was not grown.

C. moschata \times *C. Pepo*

| Parents | Number of Pollinations | Fruits | Fertile Seeds |
|--|------------------------|--------|---------------|
| Large Cheese \times Connecticut Field..... | 294 | 18 | 0 |
| Striped Cushaw \times Connecticut Field..... | 62 | 18 | 0 |
| 5 \times 175..... | 40 | 1 | 0 |
| Total..... | 376 | 37 | 0 |

The failure to secure any fertile seeds of *C. moschata* \times *C. Pepo* is very surprising, in view of the large number of fertile F_1 and F_2 seeds secured in the reciprocal pollinations.

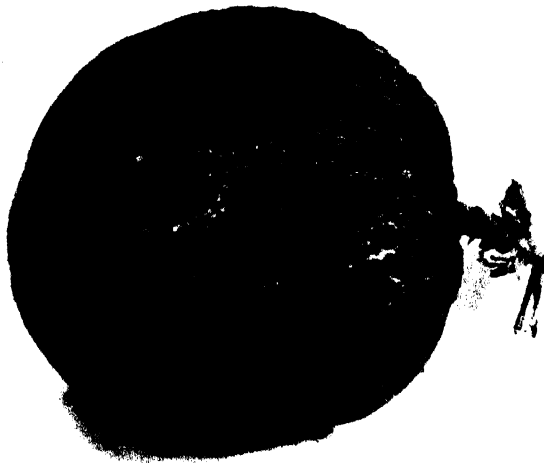
C. maxima \times *C. moschata*

| Parents | Number of Pollinations | Fruits | Fertile Seeds |
|------------------------------------|------------------------|--------|---------------|
| Hubbard \times Large Cheese..... | 3 | 1 | 0 |
| 270 \times 5..... | 2 | 1 | many |
| 20 \times 5..... | 67 | 29 | very many |

Pollination of F₁ Hybrids

| Parents | Number of Pollinations | Fruits | Fertile Seeds |
|---|------------------------|--------|---------------|
| 15 selfed | 2 | 0 | 0 |
| 15 unpollinated (tied up without pollinating) | 63 | 0 | 0 |
| 15 × 20 | 87 | 18 | few |
| 15 × 5 | 103 | 28 | few |
| 20 × 15 | 13 | 2 | 0 |
| 5 × 15 | 5 | 0 | 0 |

Of all attempts to cross species, *C. maxima* × *C. moschata* proved to be the least difficult, it being possible to secure hybrids at will. The F₁ plants of 20 × 5 (designated No. 15, text fig. 3) showed remarkable hybrid vigor.

**15**

TEXT FIG. 3. The first hybrid generation of No. 20 (inbred line of Hubbard squash) × No. 5 (inbred line of Large Cheese pumpkin), designated as No. 15.

The stems and leaves were rough hairy, leaf blades intermediate in shape between those of No. 20 and No. 5, with silvery spots at intersections of veins; pistillate flowers abundant; staminate flowers very few and sterile. Numerous fruits were secured, however, by back crossing and open polli-

nation. The fruits were nearly spherical in shape; shell thick, hard, bumpy, of uniform dark green color; fruit stalk hard, five-sided, grooved, but not flaring at attachment.

No. 18, secured by pollinating 15×20 , and No. 19, resulting from 15×5 (both back crosses) were grown in the field. It is apart from the purpose here, however, to give details concerning the characteristics of these back crosses.

C. moschata \times *C. maxima*

| Parents | Number of Pollinations | Fruits | Fertile Seeds |
|---|------------------------|--------|---------------|
| 5×20 | 8 | 2 | 0 |
| Large Cheese \times Hubbard..... | 192 | 41 | many |
| Striped Cushaw \times Victor..... | 1 | 1 | 0 |
| Striped Cushaw \times Marblehead..... | 48 | 25 | 2 |
| Total..... | 249 | 69 | many |

The small number of pollinations of the pure lines 5×20 is due to No. 5 flowering very late in the season—too late to make pollination worth while.

The F_1 of Large Cheese \times Hubbard is designated as No. 1151. This plant showed decided hybrid vigor. Leaves and stems were prickly and the leaf rather kidney shaped; spots at the intersections of the veins, however, were very suggestive of the influence of the pistillate parent. The five-sided fruit stalk was ridged and grooved and decidedly flaring at attachment to fruit. With respect to the calyx and corolla the pistillate and staminate flowers very closely resembled those of the pistillate parent. These hybrid fruits were intermediate in shape between those of both parents, being round and flattened much like the Large Cheese and resembling the Hubbard in being somewhat pointed at the blossom end. In color the fruits were dark green with lighter green pock marks—in general much like the Hubbard. The shell was medium thick and hard. Only a small number of the seeds were found to be viable, but the F_2 generation was not grown.

The two seeds resulting from Striped Cushaw \times Marblehead produced plants which were grown in the greenhouse and were very vigorous. Shape, spotting and hairiness of the leaves, as well as shape and texture of the flowers, were very similar to those of Striped Cushaw. The staminate flowers were entirely sterile, having not even a trace of pollen. Fruits were secured, however, by using pollen from a Hubbard squash growing in the greenhouse at the same time, and these fruits were identical in size, shape, and color with the Striped Cushaw itself. In no case were any viable seeds found in the fruits. Thus an F_2 generation was not obtainable.

DISCUSSION

Experimental results show that *C. maxima* and *C. Pepo* cross with each other with much greater difficulty than either crosses with *C. moschata*. It was hoped that an investigation of the chromosome numbers in the three species of *Cucurbita* would throw some light on their inter-relations, and particularly on the origin of *C. moschata*. The systematic characters of *C. moschata* are intermediate, although some of them are closely related to those of *C. Pepo*, others very similar to those of *C. maxima*. There are also a few which resemble neither *C. Pepo* nor *C. maxima*. From the standpoint of external morphology the characters of both *C. Pepo* and *C. maxima* are very distinct and clear-cut; those of *C. moschata*, on the other hand, are very difficult of demarcation. While this situation gives no clue to the origin of this plant it does strongly suggest that *C. moschata* is not to be considered as a well-defined species.

The diploid chromosome number in both *C. maxima* and *C. Pepo* was found to be forty; that of *C. moschata* forty-eight. This threw no light on the inter-relation of the species, and a cytological study of the hybrids must be made before conclusions can be drawn regarding the origin of *C. moschata*.

In planning the above investigations much thought was given the method to be used in protecting the flowers from contamination by foreign pollen. Several investigators in the field have isolated by tying a string or wrapping soft wire around the tips of the buds; others have made use of paper or parchment bags for this purpose. The writer's experience has been that perfect isolation is secured by tying the corollas if proper care is exercised; hence he chose to protect against contamination by this method rather than by the use of paper bags, as the exclusion of light and interference with the circulation of air in the latter method are very possible limiting factors in crossing species, even though this is not the case in crossing varieties.

The phenomenon of parthenogenesis reported by the Hagedoorns (12, 13), through which they secured fertile seeds from unpollinated F_1 inter-specific and intervarietal hybrids, has been reinvestigated by Lotsy. He covered a considerable number of unpollinated female flowers of inter-varietal hybrid plants with paper sacks, but in not a single case did he obtain seeds.

Vavilov (23) enclosed in parchment bags a number of female flowers of various genera and species of the Cucurbitaceae, but was unable to secure parthenogenetic seeds. It should be noted, however, that his work differed from that of the Hagedoorns in that he did not use hybrid plants. The writer made no systematic effort to study parthenogenesis in *Cucurbita*, but some data have been accumulated. With No. 20 (a pure line of Hubbard squash) 43 female flowers were tied without being pollinated.

No fruits were obtained. In No. 100 (the F_1 hybrid of 20×175) 12 unpollinated female flowers were tied, but no fruits developed. Sixty-three unpollinated female flowers of No. 15 (the F_1 hybrid of 20×5) were isolated in a similar manner, but no fruits resulted. While these data are quite meager, nevertheless so far as they go they fail to confirm the results of the Hagedoorns.

While a considerable number of hybrid seeds were secured in the above investigations, it is evident many of the pollinations produced nothing more than parthenocarpic fruits. This is in harmony with the results obtained by other workers in the field. It was observed, however, that when such parthenocarpic fruits were stored they decayed much more quickly than did fruits containing fertile seeds stored under identical conditions. An examination of the interior of parthenocarpic fruits invariably revealed the presence of many well formed seed coats which were either collapsed or inflated, and without embryos; in some cases, however, small, partially developed and undifferentiated embryos were observed, although these were too rudimentary to produce plants.

SUMMARY

1. A summary is presented of previous work on interspecific hybrids, as well as the literature dealing with the nativity of the genus *Cucurbita*.

2. Results of a cytological investigation of the three species of the genus, with special reference to chromosome numbers, are reported.

3. A summary is given of work covering a period of seven years in an effort to intercross the three annual cultivated species of *Cucurbita*.

4. Pollinating *C. Pepo* with *C. maxima* resulted in eight vigorous F_1 plants, which in turn gave rise to a large F_2 generation. Reciprocally the result was eleven fertile hybrid seeds, all the F_1 plants of which were entirely self-sterile. Fruits developed on these plants only as the result of back crossing and open pollination, and in no case did a fruit contain viable seeds.

5. Using *C. Pepo* as the pistillate and *C. moschata* as the staminate parent, fertile F_1 , F_2 , and F_3 generations were secured. It was found impossible, however, to cross *C. moschata* with *C. Pepo*, using the former as the pistillate parent.

6. A large number of successful crosses were easily made with *C. maxima* \times *C. moschata*, but the F_1 was self sterile on account of abortive pollen. It was found possible, however, to secure fruit containing viable seeds from the F_1 plants by back crossing. In the reciprocal cross more difficulty was experienced in securing viable seeds, although the hybrids were fairly numerous. A description of the F_1 generation is given.

7. Parthenocarpy was observed to be quite common, but in no instance was parthenogenesis noted.

The writer wishes to acknowledge his indebtedness to A. T. Erwin, Chief of the Vegetable Crops Section, and to Dr. I. E. Melhus, Plant Pathologist, both of the Iowa Agricultural Experiment Station, for making this investigation possible; also to Dr. J. N. Martin for valuable suggestions during the progress of the work.

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PRIMARY DORMANCY, AFTER-RIPENING, AND THE DEVELOPMENT OF SECONDARY DORMANCY IN EMBRYOS OF *AMBROSIA TRIFIDA*

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INTRODUCTION

The fruits of *Ambrosia trifida* are dormant at maturity. The dormancy is in the embryo since germination does not take place even when the embryos are freed from all enveloping structures. An after-ripening process must precede germination, but even when the seeds are after-ripened their germination, especially at high temperatures, may be considerably delayed or prevented altogether due to the fruit and seed coats. Failing to germinate under such conditions the embryos develop a second dormant condition more pronounced than the initial or primary dormancy.

Since after-ripening, germination, the development of secondary dormancy, and possibly the primary dormancy of the embryo during its period of growth and maturity on the plant are more or less influenced by these membranes, a brief description of the embryo with its accompanying structures will be given at this place.

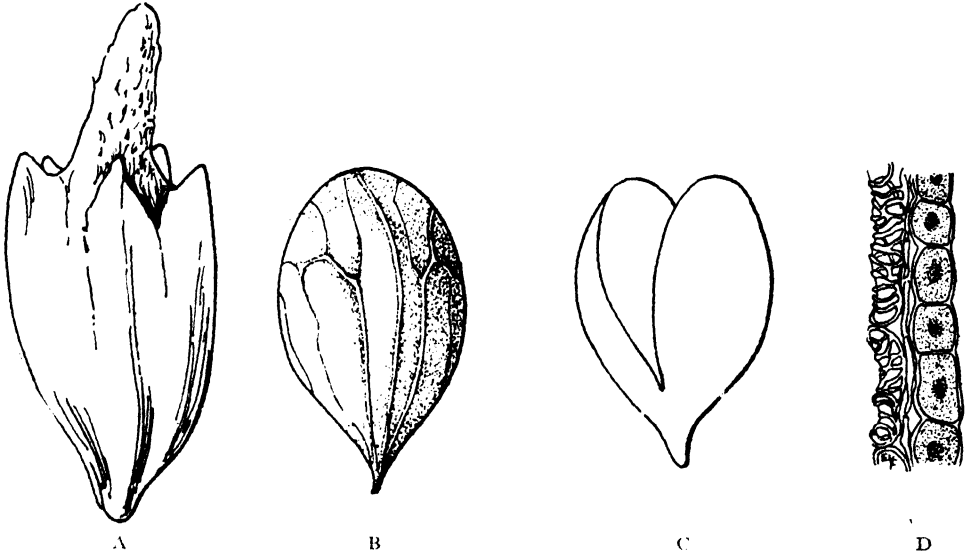
STRUCTURE OF THE FRUITS

The seed-bearing structure is a fruit (text fig. 1 *A*), the outer part of which consists of a thick-walled involucre. Beneath the involucre is the ovary, the style of which protrudes through an opening at the top of the involucre. The walls of the ovary are distinct from the involucre. Within the ovary and readily separating from it is the seed (text fig. 1 *B*). The seed consists of the embryo encased by a delicate membrane usually two layers of cells in thickness, the outer of which consists of dead cells with striated thickenings in the walls together with fragments of disintegrated cell walls constituting the remnants of the integuments. The seed coat contains numerous vascular bundles (text fig. 1 *B*). The inner layer of cells in direct contact with the embryo is of nucellar origin. It is made up of living cells with moderately thick cell walls. The entire membrane (text fig. 1 *D*) may be separated from the embryo (text fig. 1 *C*) by pressing the soaked seed between the thumb and finger. With less soaking the

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outer layer of non-living cells separates readily from the living layer leaving the embryo with only the nucellar layer intact.

As these membranes (involucre, ovary wall, and seed coats) may each be removed without interfering with the structures beneath, their combined as well as their individual effect upon after-ripening, germination, and development of secondary dormancy in the embryo may be studied.



TEXT FIG. 1. *A*, the fruit; *B*, the seed; *C*, the embryo; *D*, section of the two-layered seed coat.

In this paper three structures are considered, as shown in text figure 1: (*A*) the fruit, (*B*) the seed obtained by the removal of involucre and ovary, and (*C*) the naked embryo. Dormant embryos in all cases have been after-ripened within the fruits or within the seed. Secondary dormancy of the embryo was usually induced within the fruit and seed coats, but also within the seed coats alone.

PRIMARY OR INITIAL DORMANCY

Although all embryos are dormant at maturity, the dormancy is not equally deep seated in all, since in dry storage it may disappear early from some and late or never completely from others. The degree of dormancy of different crops varies as indicated by results reported later.

Again the dormancy is not equally pronounced in all parts of the embryo itself but is especially characteristic of the hypocotyl as has been found true of most dormant embryos, such as *Crataegus* and others (1, 2, 3). When the naked dormant embryos are placed under germinating conditions, the hypocotyls fail to elongate and no roots are produced. The cotyledons, especially those in contact with the wet medium, frequently enlarge to

several times their original size and in the light become intensely green, while those not in direct contact with the medium remain colorless and of the original size. The plumule also frequently grows while the hypocotyl remains dormant. Text figure 2 shows these characteristic behaviors of the embryos upon moist cotton in petri dishes.



TEXT FIG. 2. Dormant embryos after a period in a germinator, showing growth of cotyledons and of some epicotyls in contact with moist substratum, and no growth of hypocotyls or of cotyledons not in contact with moist substratum.

It does not seem likely that these differences in degree of dormancy especially in embryos of the same crop can be attributed entirely to conditions that obtain during storage, for while the accessibility of oxygen due to differences in permeability of enveloping membranes may not have been the same to all embryos, the temperature and the moisture content during dry storage were the same.

The primary dormancy universally present in embryos at maturity has its origin during the development of the embryo while in contact with the mother plant. Since no attempt was made to store the fruits of different crops under the same conditions any differences in their relative dormancy after similar periods of storage may have been due in part to slight differences in temperature and moisture content during storage as well as to possible differences in permeability of enveloping membranes during development on the plant for the different years.

AFTER-RIPENING IN DRY STORAGE

After-ripening or the disappearance of dormancy in embryos of fruits in dry storage, as indicated by germination, takes place slowly and very unequally in different embryos. In some, after-ripening may take place within a few months while in others the dormancy may still be present after one or more years. Excised embryos of fruits collected in 1918 when placed under germinating conditions, March 1919, gave five percent germination; in May, 36 percent, and in July, 40 percent. Excised embryos of fruits collected in 1919 gave 75 percent germination in October 1920. Embryos of fruits collected in 1924 gave 80 percent germination September 1926. Excised embryos of fruits collected in 1926 gave 40 percent germination August 1927. Embryos of fruits collected in 1927 gave no germination May 1928, while embryos of fruits collected in 1928 gave 40 percent germination July 1929. Naked embryos were used in all germination tests in order to determine whether the failure to germinate was due to the membranes surrounding the embryos or to a dormant condition of the embryos themselves. The germination tests were made at temperatures ranging between 27° and 29° C.

AFTER-RIPENING IN LOW TEMPERATURE GERMINATOR

While after-ripening may gradually go on in the embryos in dry storage, it may be brought about much more quickly and uniformly when the fruits or seeds are placed upon a wet medium such as moist cotton at a temperature of from 5° to 10° C. The optimum temperature for after-ripening at low temperature is near 5° C. and requires, for freshly harvested fruits, from 70 to 90 days. Embryos of fruits after-ripened in the cold germinated also with greater energy than embryos of fruits after-ripened in dry storage.

Whether the after-ripening takes place in dry storage or at low temperature the process is evidently the same, since fruits that have been in dry storage for some time do not require as long a period to after-ripen in the cold as do recently harvested fruits. Fruits taken from dry storage and placed upon moist cotton in petri dishes at low temperature in March required less than two months to after-ripen while still later in the season one month was frequently found sufficient for the complete after-ripening of all fruits. Embryos of fruits that had been after-ripened in dry storage and subsequently given an additional period in the cold, germinated with greater energy than those taken directly from dry storage. Many of the intact fruits after-ripened at low temperatures, germinated overnight, and the hypocotyls of naked embryos often attained a length of from one to two centimeters within 24 hours after they had been removed from the fruits and placed under germinating conditions.

RELATION BETWEEN TEMPERATURE AND MOISTURE CONTENT IN AFTER-RIPENING

Embryos of fruits kept on moist cotton in petri dishes at a temperature varying from 23 to 25 degrees from November until July gave no germination at any time during that period. When the fruits were removed from these temperatures and placed in the cold, they required fully as long a period in the cold for the embryos to after-ripen as those of recently harvested fruits. The fruits that had been in a high temperature germinator since harvest were placed in a cold germinator July 7 and on September 26, when a portion of the fruits was removed from the cold, the naked embryos gave 70 percent germination. The remaining fruits were left in the cold until November when all embryos were found fully after-ripened, giving 100 percent germination within forty-eight hours. The after-ripening of the embryos in these fruits did not take place as uniformly as that of recently harvested fruits when placed in the cold germinator.

Fruits of ragweed were planted in pots in a greenhouse October 24, 1927. No germination had occurred up to May 5 when the fruits were removed from the soil. All membranes were then removed from around the embryos and the naked embryos were placed upon moist cotton at 28° C. to germinate. None of the embryos germinated. There had been no perceptible after-ripening of the embryos during the 194 days the fruits had remained in the soil at the temperature of the greenhouse.

In a previous year fruits planted in two pots *A* and *B* in the greenhouse October 30 gave 18 and 28 percent germination, respectively, by March 7. This difference in response to soil conditions may possibly be accounted for in that the embryos of the 1927 crop were more dormant than those of the previous year, or to a condition of the embryos when the fruits were planted, or even to the temperature of the greenhouse.

While there is an optimum temperature (5° C.) for after-ripening in the cold when the fruits or seeds are in a saturated condition, there appears also to be a close relation between the temperature and moisture content during after-ripening in dry storage. Excised embryos of fruits harvested and placed over concentrated sulfuric acid in November 1926 gave no germination at any time up to August of the following year.

In April 1927 after embryos of fruits in storage had begun to after-ripen, some of these air-dried fruits were placed in a closed vessel and transferred to a refrigerator at about 10° C. The embryos of these fruits after three months did not germinate as well as the embryos of the fruits left in storage in the laboratory. Embryos of fruits stored at the same time and at the same temperature in a container in which was placed a vial containing a few drops of water in order to raise slightly the moisture content of the embryos within the fruits, after-ripened more rapidly than the embryos of air-dried fruits stored in the laboratory.

The percentage germination of naked embryos of fruits subjected to the various treatments were: fruits stored over sulfuric acid, 0 percent; fruits stored in laboratory, 40 percent; fruits stored in closed vessel in refrigerator, 25 percent; fruits stored in refrigerator with moisture content slightly above that of air-dried fruits in the laboratory, 52 percent.

There is evidently not only a minimum moisture content below which after-ripening may not take place at all or only very slowly, but there is also a close relation between the moisture content of the embryo and the temperature employed. At low temperatures after-ripening takes place most rapidly when the water content is high or when the embryos are fully saturated. At high temperatures the after-ripening of embryos in air-dried fruits takes place slowly, while in fully saturated fruits it does not take place at all. At both low temperatures and high temperatures, the moisture content of embryos determines whether after-ripening will take place and also something of the rate at which it will take place. The process of after-ripening under any conditions requires a low respiratory intensity. At high temperatures and high moisture content the processes involved in after-ripening are apparently counteracted by those involved in respiration.

THE NECESSITY OF OXYGEN FOR AFTER-RIPENING

Whatever the changes may be that take place in after-ripening, a supply of oxygen to the embryo seems necessary to initiate and complete the changes. Air-dried fruits, the soaked embryos of which gave about 40 percent germination, were sealed in jars from which the oxygen was absorbed by potassium pyrogallate. Along with these as controls, fruits were sealed in other jars from which the oxygen was not absorbed. After one month at low temperature, the embryos of fruits taken from jars without oxygen gave no greater percentage germination than the embryos of similar fruits taken from dry storage at the time the experiment was started, while the embryos of fruits taken from jars with normal oxygen pressure at the beginning of the experiment, gave a much higher percentage germination.

In order to test further the oxygen requirement for after-ripening, petri dishes were prepared with layers of moist cotton. A solution of agar was poured over this layer of cotton and when sufficiently cooled fruits and seeds were arranged upon the agar.

A thin coating of agar was then spread over the fruits and seeds in the several dishes, after which they were placed at low temperature. Along with these were other dishes containing fruits upon moist cotton only. The seeds with a very thin coating of agar after-ripened almost as readily as the fruits upon the moist cotton alone, while the fruits covered with a thicker layer of agar after-ripened much more slowly. For example, the naked embryos of fruits covered with a thicker layer of agar after three months at low temperature gave only 70 percent germination while the embryos of fruits upon moist cotton gave 100 percent germination.

CHANGES IN ACIDITY AND CATALASE ACTIVITY DURING AFTER-RIPENING

In a study of the after-ripening of seeds of *Crataegus*, Eckerson (3) found an increase both in acidity and in catalase activity.

During the after-ripening of embryos in fruits of *Ambrosia* at low temperature there was also a slight increase in acidity, probably due to hydrolysis of oils, together with a considerable rise in catalase, but it is doubtful whether either has any special significance in the process of after-ripening. Dormant embryos of fruits kept at high temperature in a saturated condition three months had an acidity slightly higher than that of air-dried seeds. Whether at low temperature or high temperature, the rise in acidity appears to be simply the result of metabolism in the embryo.

The rise in catalase, while an excellent index of after-ripening in the cold, seems also to have no special significance in the process of after-ripening, being a result and in no sense causal. Embryos of fruits in which there was a large percentage of embryos after-ripened in dry storage possessed no higher catalase content than the original dormant embryos. During the period of after-ripening in the cold there was always a considerable rise in the catalase content of embryos over that of air-dried ones. The average catalase content per embryo based upon a large number of both air-dried and after-ripened medium-sized embryos is indicated by the following data: the average cc. of O_2 released per dry intact seed was 7.2 and for after-ripened seed 11.6.

The catalase content of individual air-dried seeds of approximately the same weight may differ, as shown in table 1. This is also true of seeds after-ripened in the cold, as shown in table 2.

TABLE 1. *Catalase Content of Individual Embryos from Air-dried Fruits*

| Wt. in grams of Individual Embryos, Membranes Removed | Cc. of O_2 Released after 5 Minutes | Cc. of O_2 Released Based upon .1 gram |
|--|--|---|
| .0144..... | 5.7 | 39.5 |
| .0160..... | 5.5 | 34.3 |
| .0157..... | 5.0 | 31.8 |
| .0123..... | 5.9 | 47.9 |
| .0124..... | 5.0 | 40.3 |
| .0150..... | 6.5 | 43.3 |
| .0134..... | 5.8 | 43.2 |
| .0156..... | 6.3 | 40.3 |
| .0150..... | 5.5 | 36.6 |
| .0154..... | 5.9 | 38.3 |
| Ave. .01452..... | 5.7 | 39.5 |

The embryos of fruits in table 2 were after-ripened in an ordinary refrigerator in which there was considerable fluctuation in temperature during the period of after-ripening. On this account the catalase content of the embryos may be higher than when after-ripened at a lower constant temperature.

TABLE 2. *Catalase Content of Individual Embryos Fully After-ripened in a Low-temperature Germinator. Taken from the Same Lot of Air-dried Fruits as Employed in Table 1*

| Wet Wt. in grams of Individual After-ripened Embryos | Cc. of O ₂ Released after 5 Minutes | Cc. of O ₂ Released Based upon .1 gram |
|---|---|--|
| .0150..... | 10.0 | 66.6 |
| .0165..... | 11.5 | 69.6 |
| .0140..... | 10.0 | 71.4 |
| .0197..... | 11.5 | 58.3 |
| .0182..... | 13.0 | 71.4 |
| .0198..... | 12.2 | 61.6 |
| .0150..... | 8.3 | 55.3 |
| .0176..... | 10.3 | 58.5 |
| .0228..... | 18.5 | 81.1 |
| .0183..... | 12.3 | 67.2 |
| Ave. .01769..... | 11.76 | 66.1 |

GERMINATION OF AFTER-RIPENED FRUITS

When fruits of *Ambrosia trifida*, after-ripened in the cold, were placed upon a moist medium at a temperature between 25° and 30° C., usually a large percentage of fruits germinated within a few days, but there was always a number of fruits that did not germinate, not because the embryos within were not fully after-ripened, but because of restrictions imposed upon the embryos by membranes that surrounded them. When these membranes were removed, the naked embryos readily germinated. The percentage germination of after-ripened fruits depends upon the extent to which the after-ripening has been carried and upon the temperature at which they are placed to germinate. At temperatures around 20° C. the germination usually takes place more slowly than at temperatures near 30° C. but may extend over a longer period of time, and give a higher final percent. For example a batch of fruits was left at low temperature for three months after which they were placed to germinate, some at 30° C. and some at 22° C. After eight days 80 percent of those at 22° had germinated while only 27 percent of those at 30° had germinated. At another time two lots of after-ripened seeds of 40 each were placed at 27° and 20° C., respectively, and after seven days 52 percent of those at the higher temperature had germinated and only 35 percent of those at the lower temperature. A temperature of 30° C. is slightly above the optimum for germination of embryos inclosed within fruits.

When fruits after-ripened in the cold and especially fruits that have after-ripened in dry storage were subjected to alternating temperatures the percentage of germination was much greater than at either temperature used in the alternation, as is indicated in tables 3 and 4. In table 3 the fruits were removed from the cold as soon as the naked embryos were found capable of complete germination. In table 4 the fruits were taken from dry storage at which time about 40 to 50 percent of the naked embryos responded to germinating conditions.

TABLE 3. *The Effect of Alternating Temperatures upon Germination of Fruits After-ripened at Low Temperature*

| Number Employed | Condition | Temperature (° C.) | Percentage Germination after 12 Days |
|-----------------|-----------------|--------------------|--------------------------------------|
| 50..... | Fruits | 20 | 50 |
| 50..... | " | 20-30 | 70 |
| 50..... | " | 30 | 24 |
| 50..... | Excised embryos | 30 | 100 |

TABLE 4. *The Effect of Alternating Temperatures upon the Germination of Fruits in Dry Storage, About 40 Percent of the Naked Embryos of Which Were Capable of Germinating*

| Number Employed | Condition | Temperature (° C.) | Percentage Germination after 12 Days |
|-----------------|-----------|--------------------|--------------------------------------|
| 50..... | Fruits | 20 | 4 |
| 50..... | " | 20-30 | 24 |
| 50..... | " | 30 | 2 |

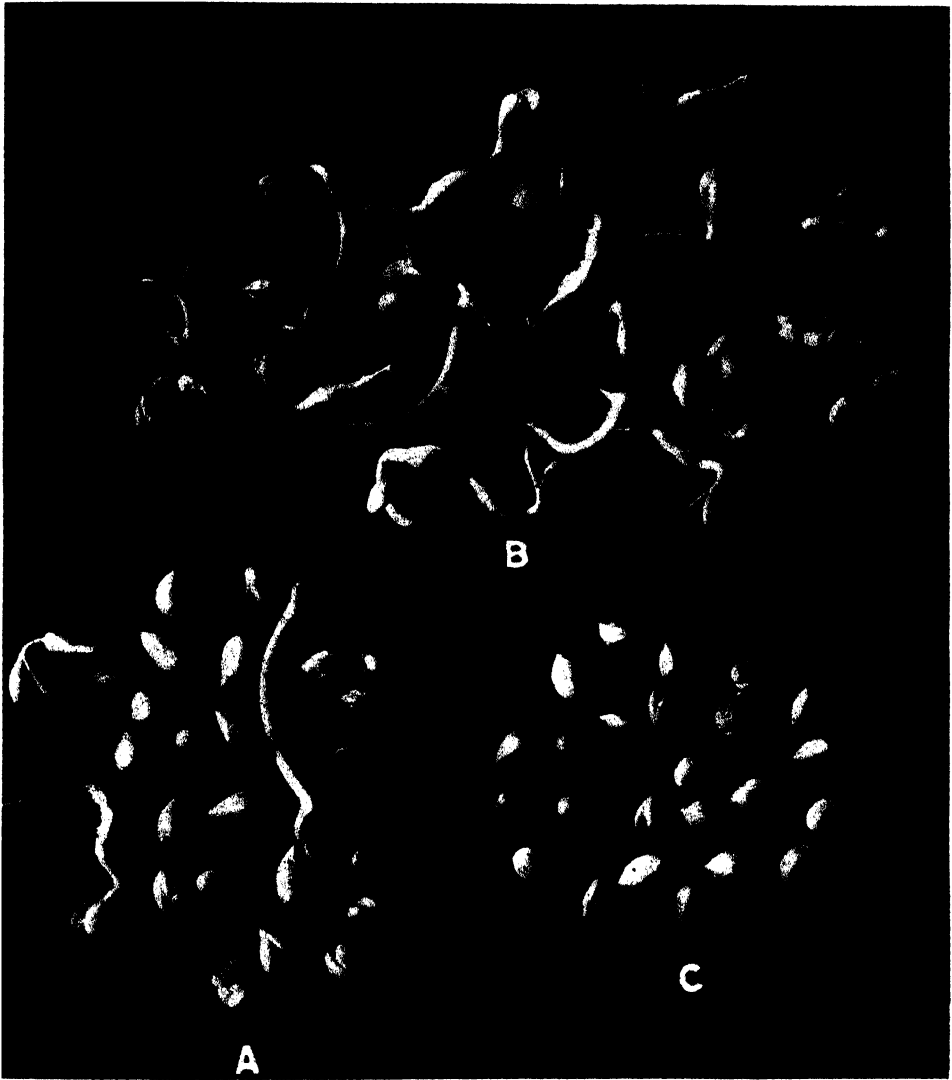
The germination of fruits evidently depends upon the degree of after-ripening, the temperature, and the oxygen available to the embryo within the fruits. The degree of after-ripening determines the capability of the embryo to respond to the conditions of germination. The oxygen supply is controlled by the membranes incasing the embryo and also by the temperature. At high temperatures an oxygen supply commensurate with the demands of the embryos cannot be maintained in all intact fruits. As a result of such limited oxygen intake embryos that are not thoroughly after-ripened or even when fully after-ripened as indicated by their ready germination when naked, are often prevented from germinating.

THE DEVELOPMENT OF SECONDARY DORMANCY IN EMBRYOS OF FRUITS AT HIGH TEMPERATURE

The after-ripened fruits or seeds of *Ambrosia trifida* that do not germinate at high temperatures do not die, but after a time revert to a dormant condition and must again be returned to the cold and go through another after-ripening process before germination can take place. It usually requires as long a period in the cold to overcome this induced or secondary dormancy in the embryo as is required to overcome the original or primary dormancy.

The time required for the after-ripened embryos within the fruit to revert to a state of dormancy in which even the naked embryos do not under any condition germinate, varied considerably. At one time fruits that required 92 days to after-ripen were removed from the cold and placed at a temperature around 28° C. to germinate. After 32 days the embryos of fruits that had not germinated were found to have reverted to the dormant

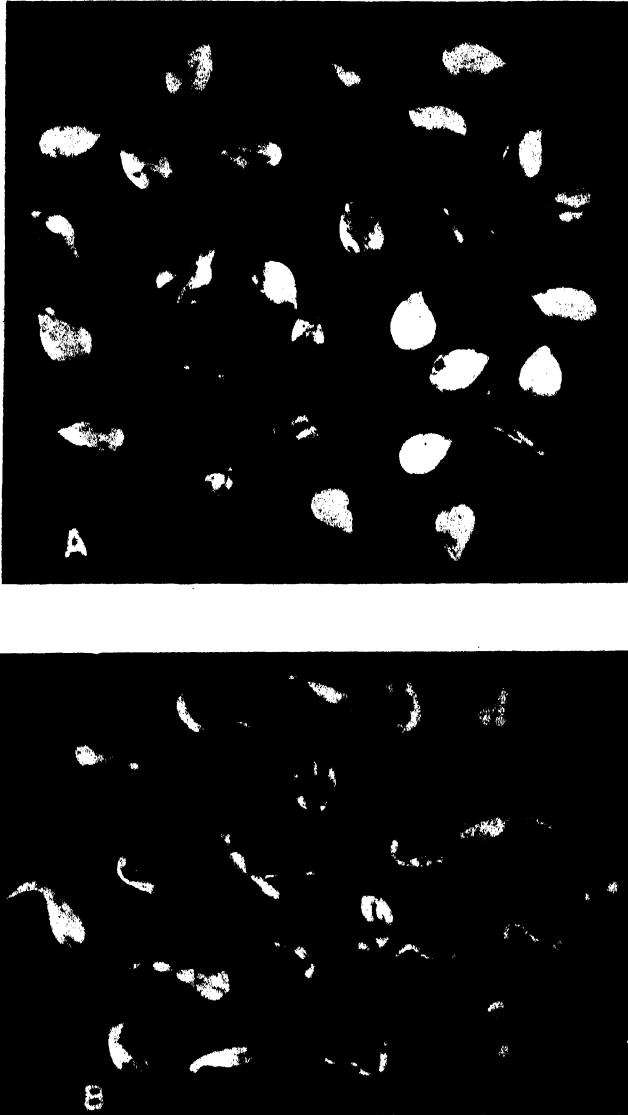
condition. These dormant fruits were again returned to the cold where they were allowed to remain 100 days, when the naked embryos were again found capable of germinating.



TEXT FIG. 3. Germination of embryos taken from fruits that had been stored for three months as follows: *A*, dry; *B*, in a germinator in a refrigerator; and *C*, in a germinator at 27°-30° C.

In July 1927 the embryos of fruits after-ripened in the cold, when placed at 29° C. for one month still gave 30 percent germination. In June 1929 both fruits and seeds, between 30 and 40 percent of the naked embryos of which would germinate, were placed upon moist cotton in petri dishes between 27° and 30° C. After 30 days, naked embryos from both fruits

and seeds were found incapable of germinating. Embryos sufficiently after-ripened in dry storage to germinate are rendered dormant more quickly than embryos of fruits after-ripened in the cold. Fruits taken from the same lot as above usually after-ripened in the cold in about a month. At



TEXT FIG. 4. *A*, embryos from seeds that have been through primary and secondary dormancy and two after-ripening periods. *B*, similar seeds after an overnight period in a germinator, showing the vigor with which they germinate.

this stage of after-ripening in dry storage the embryos within the fruits or seeds may quickly be either after-ripened or rendered dormant, depending upon the temperature to which they are subjected.

In text figure 3 are shown the effects upon the embryos of the different treatments to which the fruits have been subjected. All fruits had been in dry storage until January when they were divided into three lots. One lot was left in dry storage. A second was placed upon moist cotton in a petri dish in the refrigerator, while the third lot was placed upon moist cotton in petri dishes at temperatures between 27° and 30° C. After three months the embryos in each lot were freed from the fruits and the embryos were placed under germination conditions. Text figure 3 *A* represents the embryos of fruits in dry storage after four days in the germinator; *B*, embryos of fruits in the cold after three days in the germinator; and *C*, embryos of fruits at high temperature after four days in the germinator. No further germination took place in *A* and *C* after they were photographed.

On October 27, 1924, fruits were placed upon moist cotton in petri dishes at 5° C. to after-ripen. On February 1 of the following year when the excised embryos of all fruits responded readily to germinating conditions, the fruits were removed from the cold and placed at a temperature of 27° C. They were left at this temperature until April 29 when the embryos of the fruits that had failed to germinate up to this time were again found to have entered into a dormant condition and were returned to the cold where they were not molested until August 28, when they were again transferred to high temperature. Text figure 4 *A* shows embryos removed from fruits when taken from the cold the second time. Text figure 4 *B* shows some of these after-ripened embryos after an overnight period in a germinator at high temperature. These fruits, including the periods of time at both high and low temperatures, had spent more than 300 days in a practically saturated condition and had during that time passed through two dormant and two after-ripening periods without apparent injury.

EFFECT OF ENVELOPING MEMBRANES UPON THE GASEOUS EXCHANGE IN EMBRYOS WITHIN FRUITS AND SEEDS

Since the embryos of after-ripened fruits germinated readily when the membranes described above had been removed and since the development of dormancy is dependent upon the presence of these same membranes, their effect upon the gaseous exchange or respiration of the embryos within the fruit or the seed will be considered.

The data in table 5 show the combined effect of all the membranes taken together upon the gaseous exchange of the embryos in the fruit; the effect of the nucellar membrane alone; and the gaseous exchange that takes place in the embryos when freed from all membranes. Twenty fruits, seeds, or embryos were used in each experiment. The fruits used and the fruits from which the seeds and embryos were obtained had been in dry storage in the laboratory. No special attempt was made to select fruits, seeds, or embryos of the same weight or size, although for the same

numbers of each the weights were fairly uniform. The respiration was determined by a closed type of respirometer described by Harrington and Crocker (4).

TABLE 5. *Respiration at 30° C. after 20 Hours*

| Fruits | | | Seeds | | | Embryos | | |
|--------------------------------|---------------------------------|-----------------------------------|--------------------------------|---------------------------------|-----------------------------------|--------------------------------|---------------------------------|-----------------------------------|
| Cc. O ₂ Taken up | Cc. CO ₂ Released | CO ₂ O ₂ | Cc. O ₂ Taken up | Cc. CO ₂ Released | CO ₂ O ₂ | Cc. O ₂ Taken up | Cc. CO ₂ Released | CO ₂ O ₂ |
| 1.55 | 1.46 | .94 | 1.65 | 1.16 | .70 | 3.73 | 2.55 | .68 |
| 1.10 | 1.09 | .99 | 1.69 | 1.21 | .71 | 4.12 | 2.83 | .68 |
| 1.32 | 1.24 | .94 | 1.52 | 1.02 | .67 | 3.91 | 2.73 | .69 |
| 1.17 | 1.08 | .92 | 1.69 | 1.16 | .68 | 3.98 | 2.80 | .70 |
| 1.46 | 1.28 | .87 | 1.68 | 1.15 | .68 | 4.02 | 2.86 | .71 |
| 1.08 | .94 | .87 | 1.83 | 1.29 | .70 | 3.31 | 2.22 | .67 |
| 1.43 | 1.34 | .93 | 2.01 | 1.36 | .67 | 3.92 | 2.70 | .69 |
| 1.24 | 1.08 | .87 | 1.81 | 1.22 | .67 | 3.28 | 2.16 | .65 |
| | | | 1.59 | 1.11 | .69 | | | |
| Ave. 1.29 | 1.19 | .92 | 1.72 | 1.19 | .69 | 3.78 | 2.61 | .68 |

TABLE 6. *Respiration of Seeds and Embryos at 15° C. after 20 Hrs.*

| Seeds | | | Embryos | | |
|----------------|-----------------|-----------------------------------|----------------|-----------------|-----------------------------------|
| O ₂ | CO ₂ | CO ₂ O ₂ | O ₂ | CO ₂ | CO ₂ O ₂ |
| 1.28 | .75 | .58 | 1.88 | 1.16 | .61 |
| 1.93 | .56 | .60 | 1.82 | 1.14 | .62 |
| .77 | .50 | .60 | 2.05 | 1.25 | .61 |
| 1.18 | .69 | .58 | 1.77 | 1.09 | .61 |
| .96 | .58 | .60 | 1.92 | 1.20 | .63 |
| 1.05 | .59 | .56 | 1.94 | 1.19 | .61 |
| 1.10 | .74 | .67 | 1.59 | .99 | .62 |
| Ave. 1.08 | .63 | .60 | 1.85 | 1.15 | .62 |

It will be observed that when the membranes are removed, the gaseous exchange between the embryo and the surrounding air is increased. The nucellar membrane is more effective in reducing the volume of oxygen taken up and carbon dioxide given off by the embryo, than that of the involucre and ovary wall combined.

The nucellar membrane of the seed in text figure 1 D at 30° C. has reduced the gaseous exchange of the seed to less than one-half that of the naked embryo. It does not seem to affect greatly the respiratory ratio since it is about the same for seeds with the nucellar membrane intact as for the naked embryos.

In the fruits the respiratory ratio is higher than that obtained for either seeds or embryos. The ratio in the fruits also lacks uniformity. This lack of uniformity and possibly the high respiratory ratio may be due

to different degrees of saturation of the involucral and ovary walls or to variations in the films of water between the membranes.

At 15° C. as at 30° C. a great reduction has taken place in the volumes of the gases taken up and given off by seeds over that of naked embryos due to the presence of the nucellar membrane. The respiratory ratio is also lower than that at the higher temperature, but its value, as for the higher temperature, is practically the same for both seeds and naked embryos.

OXYGEN NECESSARY FOR INDUCING DORMANCY

While a restriction of the oxygen pressure by the membranes of seeds was found necessary for the development of dormancy, yet it does not develop in the absence of oxygen, as was found true for after-ripening. After-ripened fruits and seeds, when covered with a coating of agar by which the oxygen supply was greatly reduced, became dormant much more slowly than those not so treated. The optimum oxygen pressure in the development of dormancy, while not determined, may possibly be that pressure which at a given temperature falls just short of causing germination.

THE RELATION BETWEEN CATALASE ACTIVITY AND RESPIRATION

The catalase activity of embryos held in germinators at different temperatures with and without the various membranes, parallels closely the respiration intensity under similar conditions. The highest catalase activity is found in the naked embryos at all temperatures, and the least activity in the fruits where the oxygen supply is least. Fruits, seeds and embryos were placed upon moist cotton in petri dishes and kept at 25° C. for three days, after which the catalase content was determined for each.

In table 8 fruits, seeds, and embryos were prepared as in table 7 and placed at a temperature of 15° C. for 20 days. At the end of this period all membranes were removed from the fruits and seeds and the catalase content was determined for the three sets of embryos.

TABLE 7. *Catalase Activity after 3 Days in a Germinator at 25° C.*

| Number Used | Condition During Treatment | Cc. O ₂ Released after 10 Minutes | Average cc. O ₂ per Embryo Released |
|-------------|----------------------------|--|--|
| 3 | Fruits | 29 | 9.7 |
| 3 | " | 33 | |
| 3 | " | 25 | |
| 3 | Seeds | 43 | 14.2 |
| 3 | " | 42.5 | |
| 3 | Embryos | 70 | |
| 3 | " | 80 | 25.4 |
| 3 | " | 79 | |

In table 9 the treatment was similar to that in tables 7 and 8 except for temperature and time. They were kept at 30° C. for 10 days after which the catalase was determined on the embryos of each.

TABLE 8. *Catalase Activity after 20 Days in a Germinator at 15° C.*

| Number Used | Condition During Treatment | Wt. in grams (wet wt.) | O ₂ Released after 10 Minutes | Average cc. O ₂ per Embryo Released |
|-------------|----------------------------|------------------------|--|--|
| 2 | Fruit | .093 | 52.3 | 25.8 |
| 2 | " | .085 | 51.2 | |
| 2 | " | .085 | 51.5 | |
| 2 | " | .078 | 48.8 | |
| 2 | " | .088 | 54.5 | |
| 2 | Seeds | .090 | 59.5 | 28.5 |
| 2 | " | .087 | 62.8 | |
| 2 | " | .090 | 62.0 | |
| 2 | " | .080 | 46.3 | |
| 2 | " | .090 | 55.0 | |
| 2 | Embryos | .090 | 106.0 | 49.5 |
| 2 | " | .100 | 106.5 | |
| 2 | " | .097 | 91.5 | |
| 2 | " | .092 | 92.5 | |

TABLE 9. *Catalase Activity after 10 Days at 30° C.*

| Number Used | Condition During Treatment | Wt. in grams (wet wt.) | O ₂ Released after 10 Minutes | Average cc. O ₂ Released per Embryo |
|-------------|----------------------------|------------------------|--|--|
| 2 | Fruit | .080 | 30.2 | 14.3 |
| 2 | " | .092 | 32.0 | |
| 2 | " | .087 | 29.7 | |
| 2 | " | .090 | 25.6 | |
| 2 | " | .078 | 25.5 | |
| 2 | Seeds | .087 | 28.5 | 12.1 |
| 2 | " | .090 | 20.2 | |
| 2 | " | .085 | 19.2 | |
| 2 | " | .090 | 25.7 | |
| 2 | " | .090 | 27.5 | |
| 2 | Embryos | .092 | 51.5 | 26.2 |
| 2 | " | .093 | 47.5 | |
| 2 | " | .094 | 54.5 | |
| 2 | " | .088 | 54.0 | |
| 2 | " | .088 | 54.5 | |

At 30° C. the catalase of the embryo treated in the fruit is slightly higher than for seeds where the nucellar membrane is present. This was found true elsewhere when seeds were run at 30° C. but never true at lower temperatures.

The respiration increases as the temperature increases but the catalase of the embryos treated within the fruit or seed above a certain temperature tends to decrease. Both seem to depend upon the oxygen supply since a removal of the membranes tends to increase at about the same ratio both the respiration and the catalase content of the naked embryos over that of embryos with membranes intact. Overholser (8) found a marked increase in catalase activity in Vicar pears at 15° C., but at temperatures above that a decrease in catalase activity. Lantz (7) reported an accumulation of catalase in corn germinating at 10° C., but at higher temperatures a decrease. Somewhere between 15° and 20° C. for dormant seeds or fruits

of ragweed the catalase content for some time at least is fairly constant. Below that temperature as the embryos after-ripen there is an increase in catalase, while above it the catalase decreases. In table 10 is shown the catalase activity of fruits after 10 days at various temperatures.

TABLE 10. *Catalase Activity of Fruits after 10 Days at Various Temperatures*

| Number of Fruits | Wt. of 10 Embryos in grams (wet wt.) | Temperature (° C.) | Average cc. of O ₂ per Embryo Liberated after 10 Minutes |
|------------------|--|--------------------|---|
| 10 | .350 | 0 | 19.3 |
| 10 | .355 | 5 | 20.7 |
| 10 | .352 | 10 | 21.2 |
| 10 | .380 | 15 | 19.5 |
| 10 | .365 | 20 | 15.9 |
| 10 | .362 | 27 | 13.8 |
| 10 | .365 (air dry) | | 18 |

THE CAUSES OF DORMANCY

Since dormancy in embryos of *Ambrosia trifida* develops only at rather high temperatures, its cause seems to be due to the restricted respiration at these temperatures imposed upon the embryo by the membranes that envelop it. The nucellar membrane plays the principal rôle here. This membrane, when the seed is in a germinator at 30° C., reduces the gaseous exchange of dormant embryos to less than one half of that of naked embryos. During the period in which the after-ripened embryos become dormant there is a decrease in the intensity of the respiration in the embryos inclosed within the fruit and seed coats. The catalase content of the embryo also decreases during this time, indicating a relation between catalase activity and the respiration. Although the catalase may have no function in either after-ripening or dormancy since in either case it seems to be a result rather than a cause, yet a comparison of its activity determined from time to time upon imbibed seed at different temperatures serves as a fairly accurate indicator as to what processes are in the ascendancy in the embryo. Its rise or fall, accompanied as it is by a rise or fall in respiratory intensity, also indicates closely the temperatures at which the oxygen supply to the embryo is sufficient or deficient.

Just what takes place in the development of dormancy in seeds of *Ambrosia*, one cannot say. Kidd and West (5) were able to produce dormancy in seeds of white mustard by means of carbon dioxid, but the dormant condition tended to disappear by a removal of seed coats or a redrying of the seed. In the embryos of *Ambrosia* the induced dormancy is so deep-seated that neither redrying nor the complete removal of all membranes from the embryo will bring about germination. Partial intramolecular respiration induced at high temperatures by a restriction of the oxygen supply, together with an increase in the pressure of carbon dioxid over

that of oxygen, may bring about a sort of asphyxiation which in time may render the embryo incapable of germinating under the most favorable conditions. A slight initial rise in both the respiration and the catalase when the seeds and fruits are first placed at high temperatures followed later by a depression in each with a rising respiratory ratio indicate a partial intramolecular respiration.

On the other hand, it is possible that the restricted respiration which is necessary to produce dormancy depletes certain necessary nutrient substances or develops inhibiting substances. Since the recovery from pronounced dormancy of the embryo is brought about by subjecting the imbibed fruits or seeds to a prolonged period in a cold germinator, development of after-ripening may involve, respectively, reduction and oxidation processes in the embryo. Since oily seeds like *Ambrosia* with dormant embryos have at low temperatures a very low respiratory ratio, much more oxygen being taken up than carbon dioxide given off, some of this oxygen may possibly be fixed in the formation of more or less unstable compounds which become readily available to the embryo in germination. The rapidity and the energy which the fully after-ripened embryos exhibit in germination indicate this. At high temperatures, on the other hand, when germination is prevented by the restriction of the oxygen supply to the embryo by the enveloping membranes, oxygen may perhaps be withdrawn from compounds stored in the embryo, reducing them to more stable compounds which are not available to the embryo. The two processes, after-ripening of dormant embryos and the development of dormancy in after-ripened embryos, are opposite in nature. After-ripening involves changes, whatever they may be, that are associated with a low respiratory process which in fully imbibed embryos is attained only at low temperatures. It involves as it were a rest and a recuperation after a period of activity at high temperatures, during which the embryo has been deprived of its ability to respond to the conditions of germination.

Even after-ripening in air-dried fruits requires a considerable period of time and apparently does not take place in the embryos of some fruits at all. The after-ripening of embryos within air-dried fruits is not dependent upon the removal of water alone. Even here there is evidently some readjustment in the embryo itself, which requires considerable time. In some embryos the dormancy is so pronounced that it cannot be overcome in dry storage. It has also been demonstrated that at least some water in the embryo is necessary to induce the changes involved in after-ripening since fruits placed in a closed vessel over sulfuric acid did not after-ripen.

DORMANCY IN EMBRYO OF SEEDS IN NATURE

Many seeds are dormant at maturity; especially is this true of seeds of the *Rosaceae*. Some seeds that are dormant at maturity after-ripen under dry storage conditions, but in many the dormancy is so deep-seated

that it can only be overcome by a prolonged period in a moist condition at low temperature. The dormancy in seeds of hawthorn apparently is not diminished in dry storage and the seeds finally pass from the dormant to the lifeless condition.

As to the cause or causes of dormancy in nature one can only surmise. There may be one cause or several, but judging from the conditions under which the seeds of *Ambrosia trifida* may be made to pass in the laboratory from a condition in which they germinate readily, to a condition in which no germination will take place even with the naked embryos, one may conclude that the same causes are operating in nature as in the laboratory. Seeds in which the embryos are dormant usually mature at the end of the season so that they have a rather long period of development at high temperatures. These seeds, as far as I am aware, at least when mature, also have membranes that restrict more or less the gaseous exchange in the seed. The restriction of the oxygen supply, acting through a long period even at moderately high temperatures may bring about a state of dormancy in these embryos. In all cases where dormancy in the embryo is involved, whether produced in nature or in the laboratory, there is no doubt a very close relation between dormancy and restricted respiration.

SUMMARY

1. The embryos of fruits of *Ambrosia trifida* are dormant at maturity.
2. The embryos of the fruits after-ripen slowly in dry storage, but much more rapidly and completely in a saturated condition at low temperature (0° – 10° C.).
3. The time required for the after-ripening of freshly harvested fruits at the optimum low temperature (5° C.) is about three months.
4. During the period at which the fruits are at low temperature there is a slight rise in the acidity together with a pronounced rise in the catalase content of the imbibed embryos over those of air-stored fruits.
5. If the embryos after-ripened in either dry storage or at low temperatures fail to germinate when placed at high temperatures, due to enveloping membranes that interfere with the gaseous exchange between the embryos and the external atmosphere, they revert to the dormant condition and must again be after-ripened before germination will take place.
6. The time required for the development of secondary dormancy in the after-ripened embryos is from 30 to 60 days.
7. The same embryos of fruits may be repeatedly made dormant and after-ripened by alternating periods at high temperature with sub-minimal oxygen supply and with periods at low temperature with adequate oxygen supply in water imbibed condition.
8. The cause of secondary dormancy in embryos of *Ambrosia trifida* appears to be due to restricted respiration at high temperatures.
9. The cause of restricted respiration is the low permeability of the membranes, which envelop the embryo, to oxygen.

10. The restriction of the gaseous exchange is due mainly to the influence of the nucellar membrane, consisting of a single layer of living cells immediately enveloping the embryo.

11. While the development of dormancy is associated with restricted or incomplete respiration, the presence of a supply of oxygen seems necessary to its development. Embryos of fruits embedded in agar in order to reduce further the oxygen supply to the embryos developed dormancy more slowly than embryos of fruits at the same temperature but free from agar.

12. During the period in which the embryos become dormant both the respiration and the catalase activity are reduced.

13. At the germination temperatures employed the catalase activity of fruits, of seeds, and of naked embryos bear about the same relation to one another as does the respiratory capacity of each of these to the other.

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THE DEVELOPMENT OF DORMANCY IN SEEDS OF COCKLEBUR (*XANTHIUM*)

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INTRODUCTION

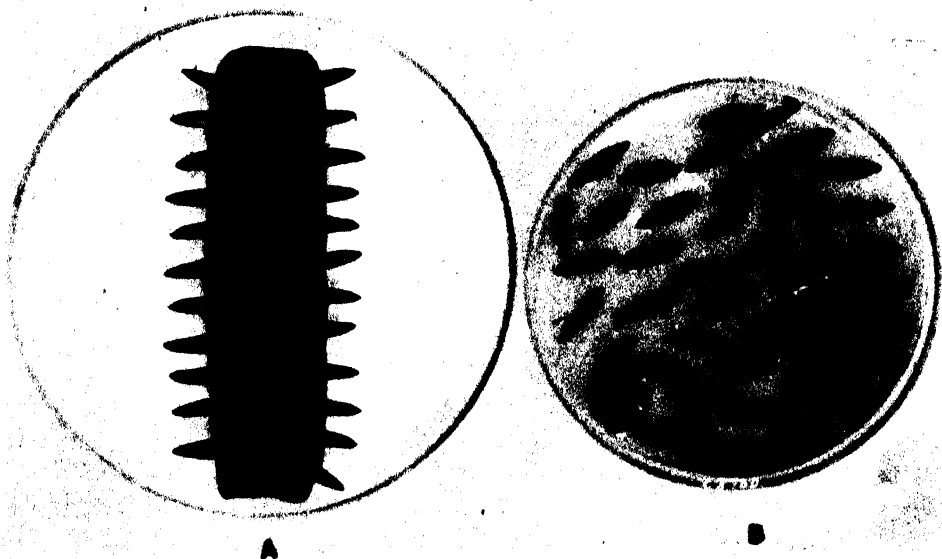
In a previous paper (2) it was shown that dormancy may be induced in the after-ripened embryos of ragweed (*Ambrosia trifida* L.) by means of high temperature germination in connection with restricted oxygen supply to the embryos due to the fruit and seed membranes that envelop them. These embryos, however, were dormant at maturity and the induced secondary dormancy was evidently merely a reversal of the essential changes through which the embryos had gone in the after-ripening process or the removal of the original or primary dormancy. The embryos of seeds of *Xanthium canadense* and *X. commune* have at no time during periods of dry storage of seeds in the burs exhibited any tendency to dormancy when placed under germinating conditions. No doubt this is also true of other species of *Xanthium*. Shull (4) compared the germination of embryos of seeds of *X. glabratum*, when quite green, with those of fully ripened seeds and of seeds one year old and was able to detect no perceptible after-ripening in passing from the unripe to the ripe and year-old conditions. The so-called dormancy or delay in the germination of these seeds at certain temperatures is due, as pointed out first by Crocker (1), to the restriction of the oxygen supply to the embryos by the seed coats for when the seed coats are removed and the naked embryos are placed under suitable conditions, germination usually takes place within 24 to 48 hours.

DEVELOPMENT OF DORMANCY IN SEEDS OF *XANTHIUM* UNDER LABORATORY CONDITIONS

Since there is this restriction of the gaseous exchange between the embryos and the outside air by the seed coats, it was thought highly probable that a dormant condition might be induced in the embryos themselves provided germination could be prevented at temperatures necessary to produce dormancy. While the restriction of the oxygen supply by the seed coats in *Xanthium* seeds is normally considerable as shown by Crocker (1) and Shull (4), it is not sufficient to prevent germination at temperatures

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necessary to produce pronounced dormancy. In order to reduce the gaseous exchange to a point where germination would not take place even at high temperatures, the hypocotyl ends of soaked seeds were embedded in modeling clay so that about one-third of the length of the upper seeds and even less of the lower seeds of burs were exposed to view. The clay with the embedded seeds was placed upon very moist absorbent cotton in petri dishes so that the exposed parts of the seeds were in contact with the wet medium. By this means seeds could be placed at once at temperatures as high as 30° C. without great loss through germination. After a few weeks in this condition, the embryos of the seeds had become sufficiently dormant to be removed from the clay and placed upon wet cotton and returned to



TEXT FIG. 1. *A*, seeds in clay in a high temperature germinator to produce dormancy; *B*, imbedded in agar for the same purpose.

the same temperature to which they previously had been exposed while in the clay. A period of from eight to ten weeks in the clay was usually sufficient to prevent germination of the seeds when transferred from the clay to the moist cotton alone.

More recently agar has been employed in preventing germination at high temperatures and has been found even more effective than clay. A solution of three or four percent agar was prepared and poured over sterilized moist cotton in petri dishes. When the solution of agar had sufficiently cooled so as not to cause injury to the seeds, the sterilized seeds that had previously been soaked overnight in water were arranged upon the agar. An additional thin layer of agar was now spread over the seeds.

The upper seeds of burs required only a very thin layer of agar to prevent germination while the lower seeds required a considerably thicker coating. Since the seeds arranged in the agar were nowhere in contact with one another, any seed that showed signs of decay while at high temperature in the incubator was readily removed without disturbing others. It was found necessary to soak all seeds before embedding in either clay or agar since the soaking brought out all defects in seed coats due to injury in the removal of the seeds from burs. All seeds with defective coats either germinated or soon decayed. Text figure 1, *A* and *B*, shows seeds embedded in clay and in agar, respectively.

Whether the dormancy was produced by embedding the seeds in agar or clay, the naked embryos exhibited many of the characteristics of dormant and partially dormant embryos as previously pointed out for dormant naked embryos of hawthorn (*Crataegus mollis*) by Davis and Rose (3) and by Davis (2) for embryos of ragweed (*Ambrosia trifida*). There was often a slight elongation of the hypocotyl, and a tendency for the cotyledon in contact with the wet medium to enlarge and become green while others not in contact with it often failed to enlarge and remained colorless. There was also a tendency for the cotyledons by unequal growth of the upper and lower surfaces to cause the embryos to become inverted upon the medium leaving the hypocotyl pointing upward. In some embryos the plumules showed slight growth while the hypocotyls remained inert.

TABLE 1. *Results with Upper Seeds Held at a Temperature of About 28° C. for 140 Days, Including the Periods in Clay and on Moist Cotton*

| | | |
|------------------------------------|---------|----|
| Number of Embryos Germinated after | 13 Days | 1 |
| " " " " " 16 " | " | 2 |
| " " " " " 18 " | " | 5 |
| " " " " " 19 " | " | 7 |
| " " " " " 24 " | " | 9 |
| " " " " " 30 " | " | 10 |

Table 1 shows the results of upper seeds of burs that were kept at a temperature of about 28° C. for 140 days, including both the period in the clay and the period the intact seeds were upon the moist cotton only. At the end of the period the seed coats were removed and the embryos were placed under germinating conditions. The number of seeds used was 11.

The remaining embryo had not germinated at the end of 30 days. The embryos of similar but untreated seeds which had been soaked 24 hours in water in order that the seed coats might be removed, gave 100 percent germination within 24 hours. The seed coats of untreated seeds as well as those of treated seeds were removed in all experiments involving germination in order to determine whether the dormancy was due to the seed coats, the permeability of which might have been altered during the treatment, or whether it was due to a condition induced in the embryos themselves.

Text figure 2 shows embryos of seeds that had been exposed to temperatures ranging from 27° to 30° C. for four months. During one-half of this time the hypocotyl ends of the seeds were embedded in clay while the remainder of that time the intact seeds were upon moist absorbent cotton



TEXT FIG. 2. Dormant embryos of cocklebur that have been in a high temperature germinator for 30 days.

only. The seed coats were then removed and the embryos returned to the incubator. The embryos were photographed 30 days later. Several of the embryos germinated during this time and were removed from the germinating chamber and so are not shown.

DEVELOPMENT OF DORMANCY IN EMBRYOS OF SEEDS OF XANTHIUM IN NATURE

Embryos of seeds collected at various times of the year from plants standing in fields at no time showed any tendency to dormancy. Since, however, a high moisture content has been shown to be an important factor in the development of dormancy in embryos one would not expect to find any marked changes in the germinating condition of such seeds. On the other hand, seeds of burs buried beneath the soil have a very different environment. Their moisture content may be high and the compact soil about the burs in which the seeds are still encased, together with that imposed by the seed coat, may materially interfere with the gaseous ex-

change of the seeds. Under such conditions, seeds that failed for any reason to germinate in the spring, would not be likely to do so later in the season, unless by some disturbance of the soil, they should be brought under more favorable conditions for germination. It is well known that of the two seeds in the burs of *Xanthium* that have been covered with soil, frequently the so-called lower seed germinates the first spring after maturity while the upper seed remains until the following spring or even later before germination takes place. This behavior has been accounted for on the basis of the difference in the gaseous exchange between upper and lower seeds (1) and the surrounding medium due to a difference in permeability of seed coats and the ability of the lower seed to germinate with a slightly lower oxygen pressure than the upper seed (4).

By removing growing plants from the soil during the summer and fall and stalks of dead plants in the spring, burs were located, the lower seeds of which had given rise to plants, but which still contained the upper seeds ungerminated. The seed coats were removed from these seeds and the embryos along with the embryos of other seeds which had been stored in the laboratory were placed under germinating conditions. The results of the germination tests are given in table 2.

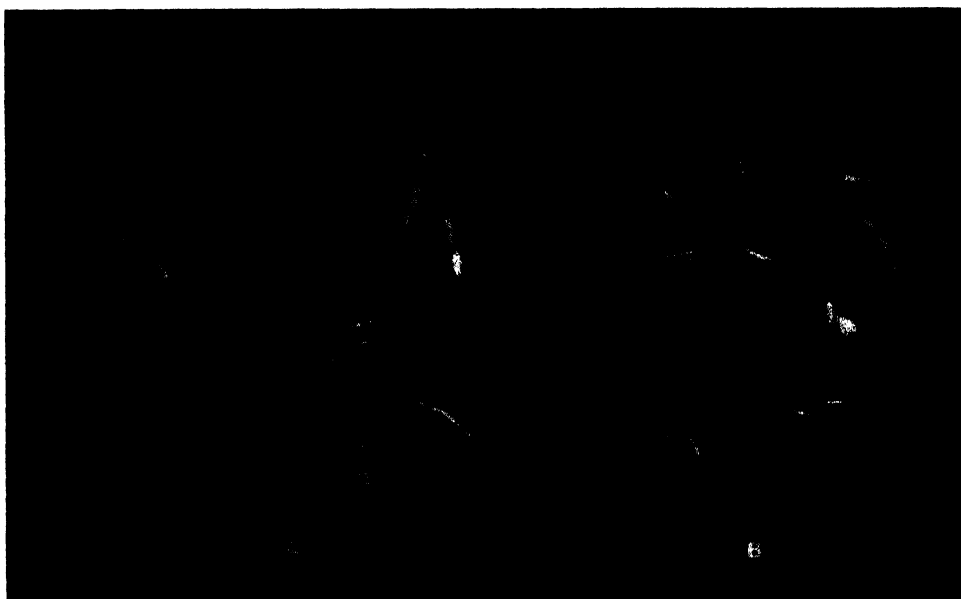
TABLE 2. *Germination of Embryos of Upper Seeds Which Were Taken from the Soil during the Summer and Fall after the Lower Seeds Had Given Rise to Plants*

| No. of Experiment | No. Seeds Used | Time Collected | Germination—Days | | | | | | | | | | | | | | |
|-------------------|----------------|----------------|------------------|---|---|---|---|---|---|---|---|----|----|----|----|----|----|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 1 | 10 | July 1 | 1 | 1 | 0 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 1 | | | | |
| 2 | 10 | " 9 | 1 | 3 | 0 | 2 | 1 | 2 | 0 | 0 | 0 | 1 | | | | | |
| 3 | 18 | " 25 | 1 | 8 | 2 | 5 | 1 | 0 | 0 | 0 | 0 | 1 | | | | | |
| 4 | 15 | " 25 | 8 | 6 | 1 | | | | | | | | | | | | |
| 5 | 20 | Control | 17 | 3 | | | | | | | | | | | | | |
| 6 | 20 | " | 20 | | | | | | | | | | | | | | |
| 7 | 15 | Sept. 7 | 0 | 1 | 0 | 0 | 0 | 1 | 3 | 2 | 4 | 1 | 1 | 1 | 0 | 0 | 1 |
| 8 | 10 | March 5 | 9 | 1 | | | | | | | | | | | | | |
| 9 | 20 | Control | 20 | | | | | | | | | | | | | | |

In experiments 1, 2, 3, and 4 the seeds were taken from a roadside where the soil was rather compact and dry at the time the seeds were obtained. In experiment 3 the seeds were kept intact in the germinator 48 hours, after which the seed coats were removed and the embryos were returned to the germinator. In experiment 4 the intact seeds were kept upon moist cotton in the refrigerator for a period of 30 days, when the seed coats were removed and the embryos were placed under the same germinating conditions as the others. In experiments 7 and 8 the seeds were obtained from a locality in which the soil was quite moist during the greater part of the year and at times was completely saturated.

In experiment 8 only a few seeds were procured from a large number of

seed stalks removed from the soil in March, due no doubt to a lack of development of a second seed in burs or to their destruction during the fall and winter by small rodents or insects. However, the rapidity or the vigor with which the naked embryos of these seeds that had passed through the winter beneath the soil germinated as compared with others under the same conditions, but collected in the fall, are doubtless characteristic of seeds that have overwintered in the soil.



TEXT FIG. 3. Embryos of cocklebur after 24 hours in germinator. A, after-ripened seeds; B, dry-stored seeds.

This shows clearly the tendency of non-germinating embryos within the seed coats and burs in the soil to go into a dormant condition during the warm weather of summer and to after-ripen, or go out of dormancy, during cool weather of winter. This development of embryo dormancy during the summer and after-ripening during the winter is probably a rather general phenomenon with non-germinating embryos of seeds of wild plants in the soil of the temperate zone. At least this rhythm has been shown in the preceding paper for seeds of *Ambrosia trifida* which have dormant embryos at maturity and in this paper for *Xanthium* seeds which have non-dormant embryos at maturity.

REMOVAL OF DORMANCY IN TREATED SEEDS

A condition of dormancy may not only be induced in the embryos of seeds of the cocklebur, but the dormant condition likewise may be removed. When the dormant seeds were placed upon moist cotton in petri dishes and kept at low temperature, preferably about 5° C., for several weeks, the

embryos gradually recovered from the dormant condition and germinated as readily or even more readily than embryos of dry stored seeds.

Text figure 3 *A* shows upper seeds of burs which were kept at high temperature in clay for four months when they were transferred to a temperature of 5° C. at which they were allowed to remain for three months. They were then taken from the cold, the seed coats removed, and the embryos placed under germinating conditions. The embryos were photographed 24 hours later. That the embryos had suffered no injury during the prolonged period at high temperature followed by an almost equal period in the cold is evident when the readiness and the energy with which they germinated are compared with conditions in embryos of dry stored seeds after a similar period in the germinator, as in text figure 3 *B*.

CATALASE ACTIVITY OF DORMANT AND AFTER-RIPENED SEEDS

The catalase activity of seeds of *Xanthium* both during the development of dormancy at high temperatures and the removal of dormancy at low temperatures corresponds to the results obtained in a study of the after-ripening and development of secondary dormancy in seeds of *Ambrosia trifida* (2).

When the seeds of *Xanthium* were prevented from germinating at temperatures at which they ordinarily germinate, through a restriction of the oxygen supply by means of clay or agar, the catalase activity decreased. Shull and Davis (5), working with *Xanthium* seeds with seed coats intact at temperatures below the minimum for germination of intact seeds, reported that at first there was a rise in the respiratory rate accompanied by a similar rise in the catalase activity, but after a period in the germinator there was a fall in each until the catalase activity was no greater than in air dry seeds.

In table 3 it will be observed that at temperatures considerably above the minimum for germination of intact seeds, but with the oxygen supply sufficiently restricted by means of either clay or agar to prevent germination, the catalase activity of seeds after from 50 to 60 days was even less than that of seeds in dry storage. The reduction of catalase of seeds with only the anterior portion embedded in clay as compared with that of the whole seed embedded in a coating of agar, is evidently due to the greater restriction of the oxygen supply to the more active portion of the embryo involving the hypocotyl and plumule. It also indicates that there is no very wide diffusion of oxygen through the embryo from the regions where it enters the seed. The respiration of intact dormant seeds was also less than that of untreated seeds. The catalase no doubt varies within certain limits with the oxygen supply to the embryo and there is a more or less close relation between the catalase activity and the restricted respiration of seeds at high temperatures as was formerly shown by Shull and Davis (5) for non-dormant seeds of *Xanthium* at temperatures below the minimum for germination.

TABLE 3. *The Catalase Activity of Xanthium Seeds under Various Conditions*

| No. of Seeds | Kind of Seeds | Treatment of Seeds | Wt. grams Soaked Seed | Cc. of O ₂ Released after 10 Minutes | Av. cc. of O ₂ Released per Seed |
|--------------|---------------|--|-----------------------|---|---|
| 2 | Upper | Seeds soaked overnight. | | 17.2 | |
| 2 | " | | | 17.0 | |
| 2 | " | | .40 | 16.5 | 8.45 |
| 2 | " | Embedded in agar 60 days at temperatures 27°-29° C. | | 14.0 | |
| 2 | " | | | 13.7 | |
| 2 | " | | .41 | 14.5 | 7.00 |
| 2 | " | Hypocotyl ends of seeds embedded in clay 60 days at temp. 27° to 29° C. | | 13.5 | |
| 2 | " | | | 10.1 | |
| 2 | " | | .42 | 11.5 | 5.8 |
| 2 | " | Embedded in agar at high temperature 80 days, then removed from agar and placed in icebox 50 days. | | 23.5 | |
| 2 | " | | | 20.0 | |
| 2 | " | | .41 | 20.5 | 10.6 |
| 2 | Lower | Seeds soaked overnight. | | 21.5 | |
| 2 | " | | | 23.7 | |
| 2 | " | | .52 | 23.5 | 11.4 |
| 2 | " | Embedded in agar 80 days at 27°-29° C., then in icebox 40 days. | | 43.5 | |
| 2 | " | | | 47.6 | |
| 2 | " | | .56 | 43.0 | 22.3 |

RELATION OF OXYGEN PRESSURE TO DORMANCY

The lower soaked seeds of burs were embedded near the bottom of large tubes of agar. The tubes were then sealed as shown in text figure 4. At this depth in the agar the oxygen supply to the embryos of the seeds must have been greatly reduced. Similar seeds in petri dishes were covered by a thin layer of agar. After 57 days at 26° to 30° C. both sets of seeds were taken from the agar, the seed coats were removed, and the embryos were placed under germinating conditions. They were photographed four days later. The results are shown in text figure 5 A, indicating the embryos of seeds taken from tubes of agar, and B, those taken from seeds in petri dishes (text fig. 1 B). The slight dormancy of the embryos taken from the tubes and the very pronounced dormancy in those taken from petri dishes can be accounted for only by the difference in the gaseous exchange of the seeds, resulting from the different depths at which they were embedded in the agar.

Seeds sealed in agar in tubes were later kept 100 days at a temperature of 30° C. without any apparent sign of dormancy in the embryos. Seeds embedded in agar at the bottom of tubes always decayed if the agar withdrew from the sides of the tubes so as to admit air.



TEXT FIG. 4. Method of embedding seeds deeply in agar.

It is probable that seeds of *Xanthium* can be kept indefinitely in a medium to which little air is admitted, provided the medium does not keep them in a so fully saturated condition as does agar, in which extreme care is necessary to prevent spoiling.

The above experiments seem to indicate that the development of dormancy, or at least the rate of development, is closely associated with the rate of oxygen supply to the embryo. Below a certain rate of oxygen supply the changes that take place during the development of dormancy either do not take place or are greatly slowed down. The same characteristic was found true of seeds of *Ambrosia* (2), but seeds of *Ambrosia*

will not stand the prolonged high temperatures to which seeds of *Xanthium* may be subjected. It was suggested in the case of *Ambrosia* that possibly a rate of oxygen supply just below that which at a given temperature is



TEXT FIG. 5. Germination of embryos after four days when taken from seeds made partly dormant by covering with agar. A, a thin layer of agar in a petri dish; B, buried deeply in agar in a test tube.

necessary to produce germination, is near the optimum for the development of dormancy.

SUMMARY

1. The naked embryos of seeds of *Xanthium* at maturity show no dormant tendencies, but dormancy may be induced in the embryos of intact seeds at temperatures at which germination ordinarily takes place, provided the restriction of the gaseous exchange by the seed coats is supplemented by means of clay or agar to a point where germination may not take place.

2. The time required for the development of dormancy in the embryos of intact seeds varies from two to several months and no doubt depends upon the temperature and on the magnitude of the restriction of the gaseous exchange.

3. During the period in which dormancy is induced there is a perceptible drop in both the catalase activity and the respiratory rate of the seed.

4. The dormant embryos of seeds of *Xanthium* after the removal of seed coats usually exhibit under germinating conditions a marked variation in the dormancy of the various embryos themselves. This is very likely due to differences in the permeability of the seed coats to gases during the period in which dormancy was induced.

5. When seeds, the embryos of which have been rendered dormant, were kept moist and at low temperatures, preferably about 5° C., the dormancy after a time disappeared from the embryos as was indicated by the rapidity or vigor of the germination of the naked embryos at suitable temperatures.

6. During the period of after-ripening or the removal of dormancy at low temperature, there was a rise in the catalase activity and also in the respiratory intensity of the seed.

7. Seeds of burs buried in the soil where they have a high moisture content apparently become more or less dormant during the summer and in turn lose this dormancy during the low temperatures of the succeeding winter and spring.

8. An oxygen supply to the seed just below that necessary to cause germination at a rather high temperature appears best for the development of dormancy.

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HASTENING THE GERMINATION OF SOME CONIFEROUS SEEDS

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Germination experiments on southern pine seeds (*Pinus taeda*, *Pinus echinata*, *Pinus caribaea*, and *Pinus palustris*) conducted in this laboratory in 1928 (Barton, 1) have been extended to include seeds of a number of different species of pine together with several other conifers. It seemed desirable to ascertain the response of these different forms to low temperature stratification which proved effective in hastening the germination of the southern pines.

The present paper reports results of these tests on both 1927 and 1928 crops of seed. Of the 1927 crop the seeds tested were: *Pinus austriaca*, *Pinus Banksiana*, *Pinus Cembra*, *Pinus densiflora*, *Pinus excelsa*, *Pinus flexilis*, *Pinus insignis*, *Pinus Laricio*, *Pinus Lambertiana*, *Pinus monticola*, *Pinus contorta Murrayana*, *Pinus ponderosa* I, *Pinus ponderosa* II, *Pinus resinosa*, *Pinus Strobus*, and *Pinus Thunbergii*. All of these seeds except those of *Pinus flexilis*, were obtained from Thomas J. Lane, seedsman, and were received in this laboratory in July 1928, when germination tests were started. They were collected in the fall of 1927 and presumably were kept in dry storage at room temperature up to the time of shipment.

The 1928 crop of seeds included *Abies arizonica*, *Cupressus macrocarpa*, *Libocedrus decurrens*, *Picea canadensis*, *Picea excelsa*, *Picea Omorika*, *Picea pungens*, *Picea sitchensis*, *Pinus austriaca*, *Pinus Banksiana*, *Pinus Cembra*, *Pinus contorta*, *Pinus contorta Murrayana* I, *Pinus contorta Murrayana* II, *Pinus contorta Murrayana* III, *Pinus Coulteri*, *Pinus densiflora*, *Pinus excelsa*, *Pinus flexilis*, *Pinus insignis*, *Pinus koraiensis* I, *Pinus koraiensis* II, *Pinus Lambertiana* I, *Pinus Lambertiana* II, *Pinus monticola*, *Pinus ponderosa*, *Pinus resinosa*, *Pinus rigida*, *Pinus Strobus*, *Pinus Thunbergii*, *Sciadopitys verticillata*, *Sequoia sempervirens*, *Taxodium distichum*, *Thuya gigantea*, *Thuya occidentalis*, and *Thuya orientalis*. *Pinus contorta Murrayana* I and *Pinus flexilis* (1927 and 1928 crops) were furnished through the courtesy of the U. S. Dept. of Agriculture Forest Service, Rocky Mt. Experiment Station, Colorado Springs, Colo. All of the other 1928 seeds were obtained from Thomas J. Lane, and most of them were received in this laboratory in November 1928.

METHOD

The method was essentially the same as that described in a previous paper (Barton, 1). Preliminary to germination experiments an examination

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of embryos was made by cutting the seeds. This process is usually called a cutting test of the embryos. These tests served as a basis for calculating "real" percentages of germination (Jacobs, 2), but they should not be considered as absolute indices of the germination capacity of the seed lot because of the limited number of seeds available for cutting. In the fresh seeds, however, there seemed to be, for the most part, a close correlation between the embryo tests and the seedling production in the soil.

The seeds were mixed with moist acid peat and placed in ovens at 0°, 5°, and 10° C. for different periods of time. They were aerated and moistened at intervals of 6 to 10 days throughout the test. In special instances other temperatures than 0°, 5°, and 10° C. were used. Some of the seeds which were very slow in germinating were given additional treatment of 15° C. as well as weekly alternating temperatures of - 5° to 5° C. and 5° to 10° C. A number of the slow germinating seeds (1928 crop) were also planted in flats and put outside in cold frames in open, mulched, and board-covered soil in December, 1928.

The number of seeds stratified depended upon the quantity available but where possible at least 600 seeds were used. Samples (100 seeds each if possible) were taken from these seeds at intervals of one, two, three, and in a few cases four or more months, and planted at a depth of 1/8 to 1/4 inches in a greenhouse in flats containing equal amounts of sand, peat, and wood soil. Samples of seeds which had been kept dry at room temperature were planted at the same time for controls.

The seedlings were counted as soon as they appeared above ground, and as a general rule they were discarded after counting. The word "germination" as it is used throughout this paper in referring to greenhouse plantings means the appearance of seedlings above ground. In describing cultures in the separate ovens the word refers to the appearance of the primary root.

Apparent germination percentages are those calculated on the basis of the number of seeds planted. "Real" germination percentages are those calculated on the basis of the number of sound embryos in the seeds used. Apparent germination percentages are used in the results and discussion except in special instances.

RESULTS AND DISCUSSION

Pinus

Pinus austriaca

From table 1 it will be seen that in the 1927 crop the cutting tests revealed 98 percent good embryos. A considerable number of these "good" embryos appeared yellow and rancid and this may account for the small germination percentages obtained. This same statement may be taken to apply in a greater or less degree to all of the 1927 seeds. This is not surprising in view of the fact that all of these seeds were practically a year

old before they were received and the vitality of most pine seeds decreases rather rapidly with ordinary storage.

TABLE I. *Results of Embryo Tests*

| Species | Crop | Number of Seeds Examined | Percent Good Embryos |
|--|------|--------------------------|----------------------|
| <i>Abies arizonica</i> | 1928 | 100 | 69 |
| <i>Cupressus macrocarpa</i> | 1928 | 100 | 59 |
| <i>Libocedrus decurrens</i> | 1928 | 50 | 40 |
| <i>Picea canadensis</i> | 1928 | 100 | 74 |
| <i>Picea excelsa</i> | 1928 | 100 | 96 |
| <i>Picea Omorika</i> | 1928 | 100 | 81 |
| <i>Picea pungens</i> | 1928 | 100 | 93 |
| <i>Picea sitchensis</i> | 1928 | 100 | 79 |
| <i>Pinus austriaca</i> | 1927 | 100 | 98 |
| <i>Pinus austriaca</i> | 1928 | 100 | 95 |
| <i>Pinus Banksiana</i> | 1927 | 100 | 97 |
| <i>Pinus Banksiana</i> | 1928 | 100 | 90 |
| <i>Pinus Cembra</i> | 1927 | 100 | 77 |
| <i>Pinus Cembra</i> | 1928 | 50 | 92 |
| <i>Pinus contorta</i> | 1928 | 100 | 99 |
| <i>Pinus contorta Murrayana</i> | 1927 | 100 | 98 |
| <i>Pinus contorta Murrayana</i> , I..... | 1928 | 100 | 98 |
| <i>Pinus contorta Murrayana</i> , II..... | 1928 | 100 | 99 |
| <i>Pinus contorta Murrayana</i> , III..... | 1928 | 100 | 98 |
| <i>Pinus Coulteri</i> | 1928 | 25 | 96 |
| <i>Pinus densiflora</i> | 1927 | 100 | 96 |
| <i>Pinus densiflora</i> | 1928 | 100 | 94 |
| <i>Pinus excelsa</i> | 1927 | 100 | 80 |
| <i>Pinus excelsa</i> | 1928 | 100 | 70 |
| <i>Pinus flexilis</i> | 1927 | 100 | 83 |
| <i>Pinus flexilis</i> | 1928 | 50 | 54 |
| <i>Pinus insignis</i> | 1927 | 100 | 97 |
| <i>Pinus insignis</i> | 1928 | 100 | 93 |
| <i>Pinus koraiensis</i> , I *..... | 1928 | — | — |
| <i>Pinus koraiensis</i> , II..... | 1928 | 25 | 100 |
| <i>Pinus Lambertiana</i> | 1927 | 22 | 100 |
| <i>Pinus Lambertiana</i> , I..... | 1928 | 25 | 88 |
| <i>Pinus Lambertiana</i> , II..... | 1928 | 50 | 78 |
| <i>Pinus Laricio</i> | 1927 | 100 | 88 |
| <i>Pinus Laricio</i> | 1928 | 100 | 100 |
| <i>Pinus monticola</i> | 1927 | 100 | 79 |
| <i>Pinus monticola</i> | 1928 | 100 | 95 |
| <i>Pinus ponderosa</i> , I..... | 1927 | 100 | 87 |
| <i>Pinus ponderosa</i> , II..... | 1927 | 100 | 94 |
| <i>Pinus ponderosa</i> | 1928 | 100 | 85 |
| <i>Pinus resinosa</i> | 1927 | 100 | 97 |
| <i>Pinus resinosa</i> | 1928 | 100 | 95 |
| <i>Pinus rigida</i> | 1928 | 100 | 91 |
| <i>Pinus Strobus</i> | 1927 | 100 | 96 |
| <i>Pinus Strobus</i> | 1928 | 100 | 98 |
| <i>Pinus Thunbergii</i> | 1927 | 100 | 85 |
| <i>Pinus Thunbergii</i> | 1928 | 100 | 79 |
| <i>Sciadopitys verticillata</i> | 1928 | 100 | 90 |
| <i>Sequoia sempervirens</i> | 1928 | 300 | 11.3 |
| <i>Taxodium distichum</i> | 1928 | 200 | 76 |
| <i>Thuya gigantea</i> | 1928 | 100 | 80 |
| <i>Thuya occidentalis</i> | 1928 | 100 | 55 |
| <i>Thuya orientalis</i> | 1928 | 100 | 74 |

* Not enough seeds to make cutting test.

The highest germination from treated seeds of *Pinus austriaca* 1927 was 16 percent which was obtained in 14 days after planting seeds which had been stratified for two months at 5° C. The average germination of the untreated seeds was 20 percent in 60 days.

Of the 1928 lot of seeds, 95 percent had good embryos and much higher germination percentages were obtained. Here the controls averaged 47 percent in 24 days. Approximately the same results were obtained from one, two, or three months' stratification at either 0° or 5° C. Seeds from these conditions gave about 50 percent germination in 12 to 18 days after planting in the greenhouse. Hence it might be said that stratification is effective in producing prompt germination of *Pinus austriaca* seeds but there is no appreciable increase in the number of seedlings.

Pinus Banksiana

Tozawa (7) reports 94 to 100 "real" percent germination for this species after the seeds had been subjected to "exposed burying storage" for a period of about four months.

Experiments in this laboratory did not give such high germination percentages (tables 2 and 3). Results indicated that the beneficial effects of stratification in this case were to be found only in the hastening of germination since the average final germination percentage of the controls was practically as high as that of the treated seeds. It would seem advisable, however, to stratify these seeds at 0° or 5° C. for a period of two months prior to planting since it makes a difference of 10 to 20 days in appearance of seedlings.

The 1928 crop of seeds showed a slight increase in germination over the 1927 crop but this difference was not so marked as in the case of *Pinus austriaca*.

Pinus Cembra

Although germination tests were made on both 1927 and 1928 lots of these seeds, no seedlings were obtained. The cutting tests revealed many embryos which apparently were sound but which had a wrinkled and rather dried appearance. It is possible that none of these seeds were viable, but it is very likely that favorable after-ripening conditions have not yet been found.

Kienitz (3) found that Cembra pine under the most favorable conditions in a seed bed rarely germinates the first year but there is abundant germination the second year. Zederbauer (9) also reports that *Pinus Cembra* and *Pinus koraiensis* proved especially hard to germinate. It is certain that the germination of *Pinus Cembra* seeds will require a great deal more study.

Pinus contorta

Of this species Toumey and Stevens (6) say: "The Pacific coast form of this species and the Rocky Mountain form known as lodgepole pine show,

in the nine tests from seeds collected in different regions and at different times, a remarkably low germination within the period of the test. In one sample but 14 percent germinated within the period of 50 days, yet cutting tests at the time of termination of the experiment showed that 80 percent of the ungerminated seeds were sound. The average germination in 50 days was only 11.2 percent and the highest 24 percent."

All the seeds available for this study were of the 1928 crop. Ninety-nine percent of the seeds were apparently good. The response to stratification was definite and favorable. The optimum temperature for stratification seemed to be 5° C. and the time two months. Seeds planted after this treatment gave 86 percent germination in 18 days whereas the average for the checks gave 51 percent in 28 days.

Pinus contorta Murrayana

Although 98 percent of the 1927 crop of the seeds of this species contained embryos, the results of the germination tests indicated very low vitality, especially so when compared with the results of three lots of the same kind of seed from the 1928 crop. The highest germination percentage (6 percent) was obtained from seeds which had been stratified for two months at 5° C. The check lot of seeds averaged four percent germination so it cannot be said that stratification had any appreciable effect.

There was, however, a decidedly favorable response to stratification in the 1928 seeds of which there were three different lots, numbers I, II, and III. Ninety percent of the seeds of number I germinated in 14 days after two months' stratification at 5° C. while the average control for these same seeds gave 63 percent germination in 24 days. Similar results are to be found in the cases of numbers II and III.

For *Pinus contorta Murrayana*, then, stratification of the seeds at either 0° or 5° C. for one, two, or three months is decidedly beneficial in the production of prompt and complete stands of seedlings.

Pinus Coulteri

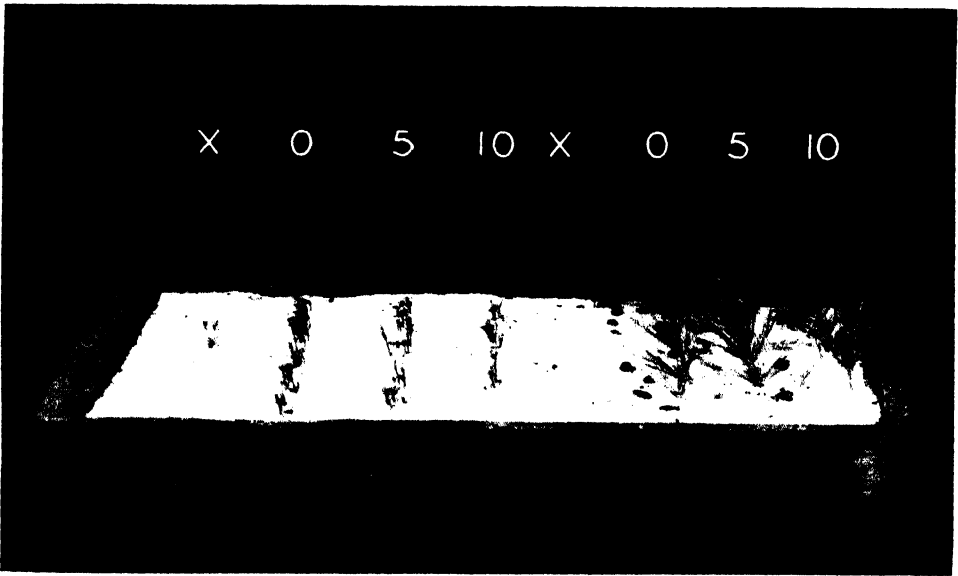
Only a limited number of the 1928 crop of these seeds was available and yet they furnished one of the most striking examples of low temperature stratification effects.

The sample plantings were of ten seeds each. Seeds which had been stratified at 0°, 5°, or 10° C. for one, two, or three months germinated to the extent of 80 to 100 percent in 19 to 25 days after planting in a flat in the greenhouse (text fig. 1, right). None of the untreated seeds had germinated at the end of 50 days.

Pinus densiflora

In this instance there was very little difference in the results of the germination tests on 1927 and 1928 seeds. The cutting tests also showed that the two lots were of practically equal vitality.

Stratification for two months at 0° or 5° C. proved effective in hastening germination. The seeds left at these temperatures longer than three months began to germinate. This was especially true of the 1928 seeds. A small percentage germinated at 10° C. before they had been in this condition one month. However, a sample planting of 1928 seeds after stratification for one month at 10° C. resulted in the best germination percentage (82) obtained in these tests. Hence it would seem that there would be very little if any loss due to seedling production in the oven at 10° C. and at the same time good results could be obtained from greenhouse plantings after one month at this temperature. It would be impractical to stratify at 10° C. for a longer period.



TEXT FIG. 1. Seeds were stratified for one month at 0° , 5° , and 10° C. Picture taken 25 days after planting. Left to right: *Thuja gigantea*; control, 0° , 5° , and 10° C., *Pinus Coulteri*; control, 0° , 5° , and 10° C.

Tozawa (7) reports 98 to 100 percent "real" germination of *Pinus densiflora* after four or five months' "exposed burying storage" or "indoor burying storage." He also obtained 99 to 100 percent "real" germination from one month (March) "exposed burying storage" and from dry storage. However, he found that the seeds with four or five months' treatment germinated more readily.

Pinus excelsa

This species of pine proved one of the most difficult to germinate. The best germination was eight percent in 25 days. This resulted from a planting of 1928 seeds which had been stratified at 10° C. for three months. In addition to the usual tests after one, two, and three months at 0° , 5° ,

and 10° C., plantings of the 1927 seeds were made after four and eight months at 5° C. There was no germination from the former and only one percent in 15 days from the latter. Seeds of the 1928 crop were tested after four months at 0°, 5°, and 10° C. Those from 0° C. germinated to the extent of two percent 25 days after planting in the greenhouse.

Additional seeds of the 1928 crop were stratified at an alternating temperature of - 5° to 5° C. and at a constant temperature of 15° C. for periods of one, two, three, and four months. Sample plantings were made from each temperature at the end of each of these periods but no seedlings were produced.

Since the cutting tests revealed 80 and 70 percent good embryos in the 1927 and 1928 lots, respectively, it is evident that the methods here reported are unsatisfactory for the treatment of seeds of *Pinus excelsa*.

Pinus flexilis

A reference to table 1 will show that in this case the 1927 seeds were better than the 1928. In either case the seeds may be left at 0°, 5°, or 10° C. for one, two, or three months. The response of the 1927 seeds to any of these conditions was very marked. For instance, after two months at 5° C., a sample planting gave 100 percent germination 18 days after planting, while the control produced 50 percent germination in 40 days.

The stratification effects were not so striking for the 1928 seeds (tables 2 and 3). Here the principal result was the shorter germination period of treated seeds, as the final germination percentages from untreated seeds approximated the number produced from treated seeds.

Pinus insignis

As would be expected from the results of the cutting tests (table 1) 1927 and 1928 crops of these seeds behaved in a similar manner. For both of them 5° C. for two or three months was favorable for after-ripening. Seeds thus treated produced a rather complete stand of seedlings (75 to 84 percent) in 14 to 18 days while the untreated seeds required 50 to 70 days for a smaller percentage germination (69 percent).

Pinus koraiensis

No 1927 seeds of this pine were available but experiments were performed with two different lots of 1928 seeds. From table 3 it will be seen that the first lot (*Pinus koraiensis* I 1928) gave 20 percent germination within 25 days after planting when the seeds had been stratified for three months at 5° C. Seeds of *Pinus koraiensis* II 1928, however, did not germinate at all when planted after stratification at 0° or 5° C. for one, two, or three months, but produced seedlings to the extent of ten percent after stratification for one or two months at 10° C. When these seeds were planted after stratification for five months at 0° or 5° C., 33 percent of

them germinated in 43 days. This seems to point to the need of a long stratification period.

Seeds of *Pinus koraiensis* were also stratified for one, two, and three months at a constant temperature of 15° C. and at a weekly alternating temperature of - 5° to 5° C. Results of sample plantings showed these temperatures to be of little value for stratification.

In all sample plantings of stratified seeds of this species only ten seeds were used in each lot.

Samples of one hundred seeds each of *Pinus koraiensis* I 1928 were planted in flats and put in cold frames in mulched, board-covered, and open soil on December 10, 1928. On July 2, 1929, seeds held under the three conditions had germinated to the extent of 12, 21, and 6 percent, respectively.

Further experiments on *Pinus koraiensis* are now being planned.

Pinus Lambertiana

In spite of the fact that embryo tests of *Pinus Lambertiana* II 1928 showed that the seeds of this lot were inferior to those of *Pinus Lambertiana* I 1928 or of *Pinus Lambertiana* 1927 (table 1), the highest germination percentage (92) was obtained with these seeds after they had been stratified for three months at 10° C. Each of the three lots, however, showed the marked effect of low temperature stratification as an effective agent for hastening germination. This fact is clearly shown in tables 2 and 3 and is significant in view of the difficulties in germination reported by Toumey and Stevens (6). They say that the earliest germination attained in any of the tests was 20 days. In some of their samples there was no germination in 50 days. The germination in 50 days averaged but eight percent.

Jacobs (2) reports the normal germination of sugar pine based on 12 tests for 120 days as varying from 18 to 53 percent. Soaking for four days and exposure to freezing for 48 hours he found most favorable to induce the early and complete sprouting of sugar pine seeds. The beneficial action of soaking, Jacobs considered due to the action of bacteria in tap water exposed to air. This is recommended as a possible means for obtaining a high percentage of vigorous seedlings in nursery beds. He found soaking for four days most beneficial of all pretreatments tried and in his lots 1 and 4 the germination reached 80 and 73 percent ("real" germination percent), respectively, in a period of 20 days. He used fresh seeds, that is, extracted seeds which had been dried for one week at an average room temperature of 22° C. before being used for germination tests.

Stratification effects on *Pinus Lambertiana* II 1928 here reported compare favorably with the germination results of Jacobs (2). After three months at 0°, 5°, and 10° C. these seeds produced seedlings to the extent of 92, 85, and 118 "real" percent, respectively (table 2). A "real" percentage of over 100 is due either to the planting of seeds which have more than the

average number of good embryos or to the selection of poor seeds for the cutting tests.

Pinus Laricio

For the 1927 seeds one or two months at either 0° or 5° C. puts the seed in condition for sprouting. Here the beneficial effect of low temperature treatment is to be found only in the earlier stands of seedlings since the untreated seeds germinated equally well but in 60 days as compared to 14 to 18 days for the treated seeds.

The 1928 seeds were not received until May 1929, and seem to have poor germination quality (tables 2 and 3). However, the stratification effects are noticeable (table 3).

Pinus monticola

Of the seeds of this species Toumey and Stevens (6) say: "The earliest germination was in 25 days. The highest number of sound ungerminated seeds after 50 days was 48 percent. In this sample no seeds germinated within the 50-day period. Taking these samples as a whole they show a remarkably low germination capacity. Furthermore, the germination in 50 days averaged but 2.7 percent."

Wahlenberg (8) has worked with these seeds and reports that fall sowing of western white pine results in prompt and complete germination the following spring. Larsen (4) reports the same beneficial effect of fall sowing.

The 1927 and 1928 lots of seeds used in the present study had 79 percent and 95 percent good embryos. Consequently there should have been a better yield of seedlings from untreated seeds than that reported by Toumey (6). We have obtained similar results. The average percentage obtained after 50 days in the 1927 lot was 11 and that obtained from the 1928 seeds was ten in the same period.

In the 1927 seeds, the best germination obtained was 48 percent. This resulted 70 days after planting seeds which had been stratified for three months at 5° C. However, the major portion of these seedlings had been produced at the end of 37 days.

The best germination obtained in the 1928 seeds (30 percent 23 days after planting) was the result of three months' stratification at 0° C.

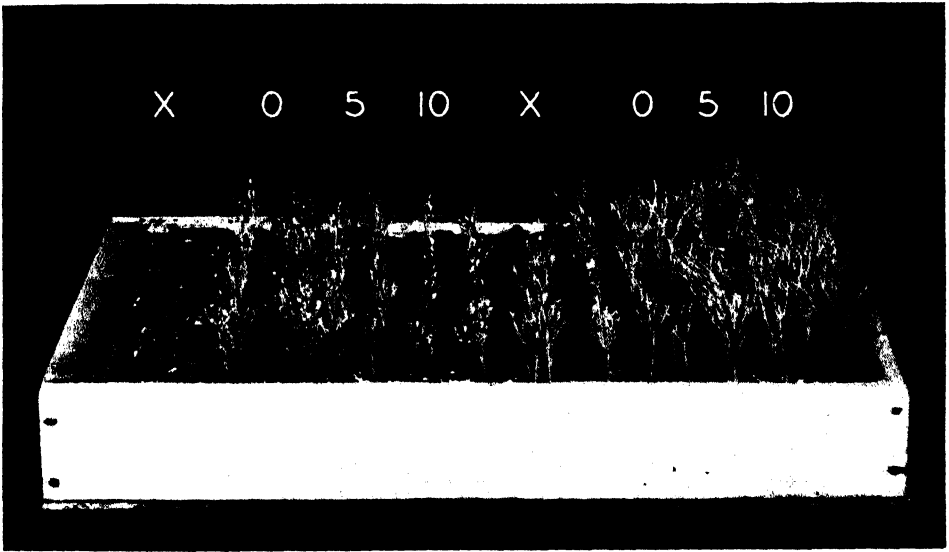
Four and five months' stratification at 0°, 5°, and 10° C. as well as one, two, three, and four months at a weekly alternating temperature of 5° to 10° C. were also tried with the 1928 seeds. None of these results were superior to those already given.

Three flats of 1000 seeds each were planted and put in cold frames in mulched, board-covered, and open soil December 10, 1928. On July 2, 1929, these flats showed germinations of six, eight, and seven percent, respectively. The number of seedlings obtained in this experiment was unduly low because part of the seeds were eaten by mice.

Pinus ponderosa

Toumey and Stevens (6) report an average germination of 43 percent in 50 days with the earliest germination within ten days after starting the tests. They found that some samples attained a germination as high as 22 percent in ten days and that the germination energy period was usually within 25 days and sometimes within 15 days after seeding (text fig. 2, right).

Both lots of 1927 seeds used in the present experiments yielded better productions of seedlings than the 1928 seeds. In general *Pinus ponderosa*



TEXT FIG. 2. Seeds were stratified for two months at 0°, 5°, and 10° C. Picture taken 19 days after planting. Left to right: *Pinus resinosa* 1928; control, 0°, 5°, and 10° C. *Pinus ponderosa* 1928; control, 0°, 5°, and 10° C.

seeds germinate readily without treatment of any kind. In every case the untreated seeds had begun to germinate in ten days and in one case (*Pinus ponderosa* II 1927) the germination within this period amounted to 36 percent. This is in agreement with Toumey's report as is the fact that the germination is practically complete within 20 to 30 days. However, a higher final germination percentage (50 to 79 percent) was obtained in the present experiments.

Stratification for one or two months at either 0° or 5° C. has the advantage that a higher germination percentage (74 to 97 percent) is promptly obtained (tables 2 and 3).

Pinus resinosa

Both the 1927 and 1928 seeds responded favorably to stratification for one, two, or three months at 0°, 5°, or 10° C. Very good germination (from 73 to 95 percent within a period of 14 to 25 days) was obtained in

all cases (text fig. 2, left). However, the control lots of seed also produced good stands of seedlings (64 to 67 percent in 20 to 24 days).

In tests on *Pinus resinosa*, Toumey and Stevens (6) obtained an average germination of 33.6 percent in 50 days. They also report that taking the samples of this species as a whole, the peak of germination was attained in 25 days or less.

Pinus rigida

Only 1928 seeds of this species were tested. Table 3 reveals very clearly the beneficial effect of stratification. Here it is seen that one month at 5° C. gave as good results as two or three months. Seeds planted after one month at 5° C. gave 87 percent germination after 12 days and 95 percent after 18 days and if the experiment was extended to 40 days the percentage germination reached 99. This is in sharp contrast to the untreated seeds of which only three percent had germinated after 12 days and which reaches 33 percent after 50 days (text fig. 3).

Very good germination (79 to 96 percent) was also obtained after one, two, or three months at 0° or 10° C. (table 2).

Pinus Strobus

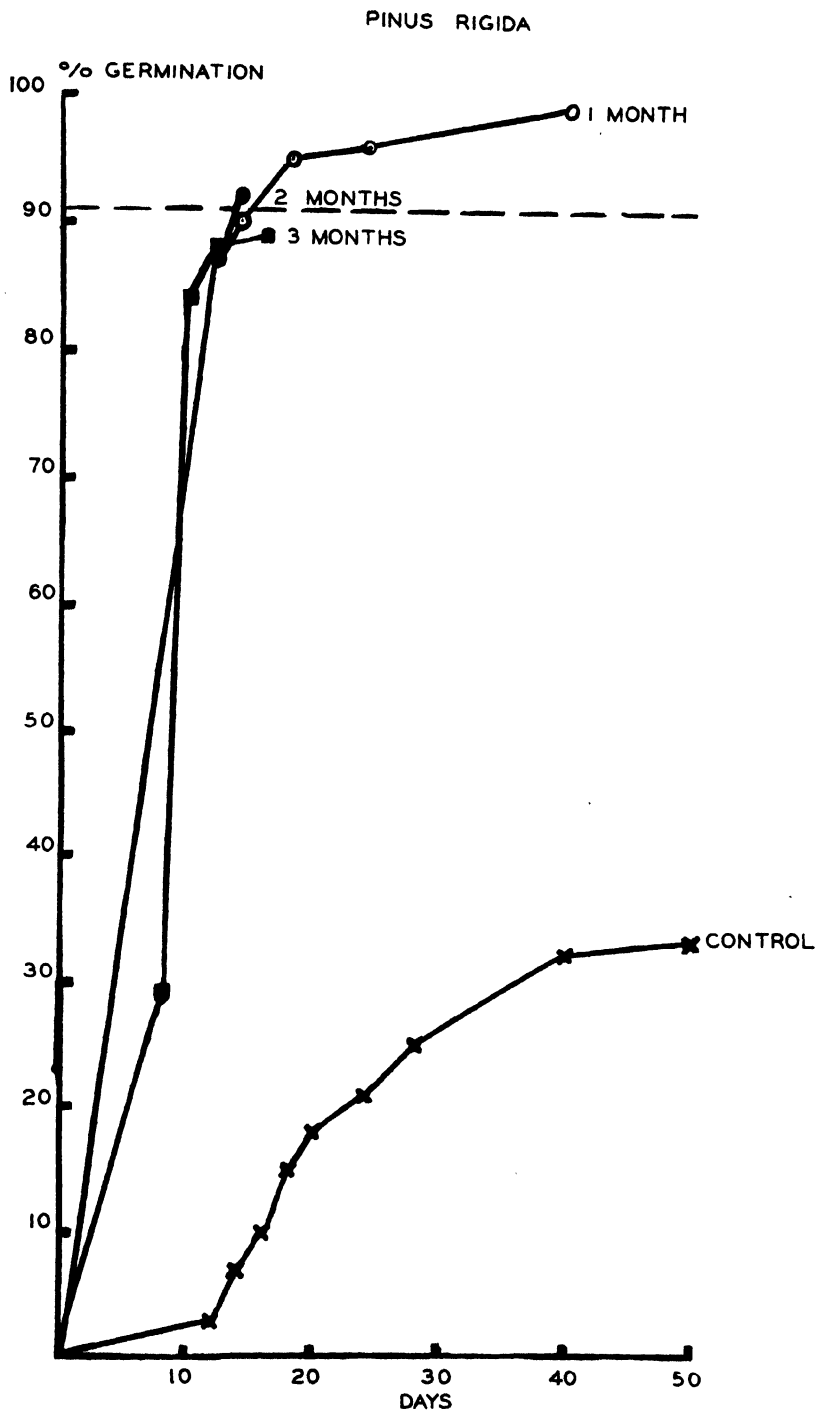
The seeds of this species proved rather difficult to germinate. In spite of this fact, however, the low-temperature effects were quite marked and especially so in the 1927 seeds. For instance after two months at 5° C., 69 percent of the seeds had germinated 24 days after planting while the corresponding control showed three percent germination 60 days after planting (see table 3).

After the same stratification period the 1928 seeds attained 26 percent germination in 24 days, while the corresponding control yielded four percent 28 days after planting.

Seeds were also stratified at a weekly alternating temperature of - 5° to 5° C. as well as a constant temperature of 15° C. From these stratifications sample plantings were made after one, two, three, and four months. The results showed these temperatures inferior to those of 0°, 5°, and 10° C. or after-ripening.

Pinus Strobus seeds have been reported as being very irregular in germination and as being greatly benefited by fall sowing (Toumey, 6), which practice is, of course, essentially the same as low-temperature stratification.

According to Schmidt (5), *Pinus Strobus* stored dry will not germinate without soaking in water. He advises storage in a cool, moist room for 30 days after a swelling period of 16 hours. As a result of treatment with a 16-hour water bath, he reports 78 percent germination. He also soaked the seeds in one percent hydrogen peroxid for 16 hours. From these seeds he obtained 81 percent germination in 60 days.



TEXT FIG. 3. The effect of stratification at 5° C. for one, two, and three months on germination of the seeds of *Pinus rigida*. Dotted line shows the percentage of good seeds as revealed by embryo tests.

Pinus Thunbergii

Seeds of both 1927 and 1928 crops proved difficult to germinate. The highest germination obtained was 20 percent. Five degrees C. proved as good as any temperature for after-ripening these seeds. When one compares the percentage and time of germination of the stratified and untreated seeds (table 3), the benefit of the low-temperature treatment is apparent. However, it leaves much to be desired.

One thousand seeds each were planted in three flats which were put in cold frames December 10, 1928, in mulched, board-covered, and open soil. On July 2, 1929, seedlings had been produced to the extent of two, four, and five percent, respectively.

Other Coniferae*Abies arizonica*

Cutting tests showed that 69 percent of the seeds had good embryos. The best germination was 43 percent which was obtained 41 days after planting seeds which had been stratified for one month at 0° C. (table 2). However, seeds which had been stratified for two months at 5° C. produced seedlings to the extent of 36 percent within 14 days after planting. Since the control gave only two percent germination after 50 days, the beneficial effect of low-temperature treatment is evident.

Cupressus macrocarpa

Toumey and Stevens (6) found that the highest germination in 50 days was 17 percent and the average 9.7 percent. They report the highest germination capacity as 37 percent and the average only 16.2 percent.

In spite of the fact that cutting tests showed a germination capacity of 59 percent, the average germination of untreated seeds in the present tests was only six percent after 50 days (table 3). However, higher percentages were obtained after low temperature treatment. The best germination was 22 percent which resulted 23 days after planting seeds which had been stratified at 0° C. for two months. The best stratification temperature tried for these seeds was 0° C. (table 3).

Libocedrus decurrens

A small number of 1928 seeds of this species were received in May 1929. Cutting tests revealed only 40 percent good embryos.

The highest germination obtained was 23 percent (58 percent real germination) which resulted 24 days after planting seeds which had been stratified for one month at 0° C. These seeds also responded well to two months' stratification at either 0° or 5° C. (table 2). In any case low-temperature treatment had a marked effect since the control lot of seeds had germinated to the extent of only one percent in 50 days.

Picea canadensis

Stratification at 0° C. proved more favorable for after-ripening these seeds than at 5° C. while 10° C. could not be used at all because too many seeds (16.4 percent) germinate within a month at this temperature. From table 2 it will be seen that the highest germination percentage (96 percent in 25 days) was obtained after the seeds had been kept for two months at 0° C. The untreated seeds germinated to the extent of 48 percent in 26 days (table 3). Toumey and Stevens (6) report 37 percent as the highest germination percentage obtained in 50 days.

Only 1928 seeds of this species were available for the present tests.

Picea excelsa

The response of these seeds to low temperature stratification was essentially the same as that of *Picea canadensis* (tables 2 and 3).

Picea Omorika

In this species stratification for one or two months at 0°, 5°, or 10° C. appears to be equally good. The highest germination was 66 percent within 15 days after planting. In view of the fact that cutting tests revealed only 81 percent good embryos, the above percentage represents fairly complete germination. The average control gave ten percent germination in 16 days.

Picea pungens

In this case one month's treatment at 0°, 5°, or 10° C. is sufficient to give a stand of seedlings of from 74 to 80 percent in 16 days (table 2). The germination of the untreated seeds was only 47 percent in 50 days.

Picea sitchensis

This species proved more difficult to germinate than the other species of *Picea* included in this study. Of these seeds Toumey and Stevens (6) observed that germination seldom gets well under way for a period of 20 to 30 days and usually many sound seed remain ungerminated after a period of 50 days.

This same observation was made in the present study. However, since seeds which had been stratified for two months at 5° C. yielded 24 percent seedlings in 27 days and the control yielded only four percent in 40 days, again we can say that although we do not obtain a complete stand of seedlings the beneficial effect of stratification is evident. The germination tests were allowed to continue for 60 days.

Sciadopitys verticillata

Sciadopitys verticillata seeds were obtained from Conyers B. Fleu, seedsman, and were received in this laboratory January 2, 1929. The seeds were of the 1928 crop and were originally from Japan.

Germination tests of these seeds were made preliminary to storage tests which are now in progress. Oven tests of 200 seeds each were made in peat at constant temperatures of 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. as well as daily alternating temperatures of 10° to 30° C., 15° to 30° C., 20° to 30° C., and 20° to 35° C. The best germination temperature proved to be 20° C. where a percentage of 52.5 was obtained in 61 days with a final percentage of 60.5 in five months. The period of these tests was seven months.

Stratification of these seeds differed from that of any other seeds in this report in that three different media (peat, leached peat, and muck) were used. One thousand seeds each were placed in each medium at constant temperatures of 0°, 5°, 10°, and 15° C. as well as daily and weekly alternating temperature of 5° to 10° C. At the same time (January 1929) 1000 seeds each were planted in five flats, one of which was placed under each of the following conditions: 1, open cold frame; 2, mulched cold frame; 3, board-covered cold frame; 4, lowest temperature greenhouse available (40°–45° F.); and 5, highest temperature greenhouse available (65°–70° F.). The greenhouse temperatures increased, of course, as the season advanced.

Sample plantings from all the stratifications were made after two, three, four, five, and six months. The seedling productions from 0°, 5°, and 10° C. for one, two, and three months are shown in table 2. The best stratification conditions found were 10° C. for one or two months, or 0° or 5° C. for two months. No seedlings have as yet been obtained from the fourth, fifth, or sixth month's planting. However, since the seedlings do not begin to appear until 60 to 70 days after planting and since these last plantings were made in June, July, and August, 1929, it is possible that the seedling production will indicate some advantage in stratification.

In view of the results of the tests it would seem that stratification has no particular effect in hastening the germination of seeds of *Sciadopitys verticillata* since the untreated seeds grow just about as well as the treated ones (table 3).

Of the seeds which were planted in flats, those in the warm greenhouse germinated first. Here the germination reached 13.6 percent in three and one-half months and in four months the percentage was 27.3. After four months this flat was badly infected with damping-off which doubtless precluded additional germinations. Seedlings appeared in the low-temperature greenhouse after seven months (7.5 percent), while the seeds in the cold frames had produced no seedlings in this period.

Sequoia sempervirens

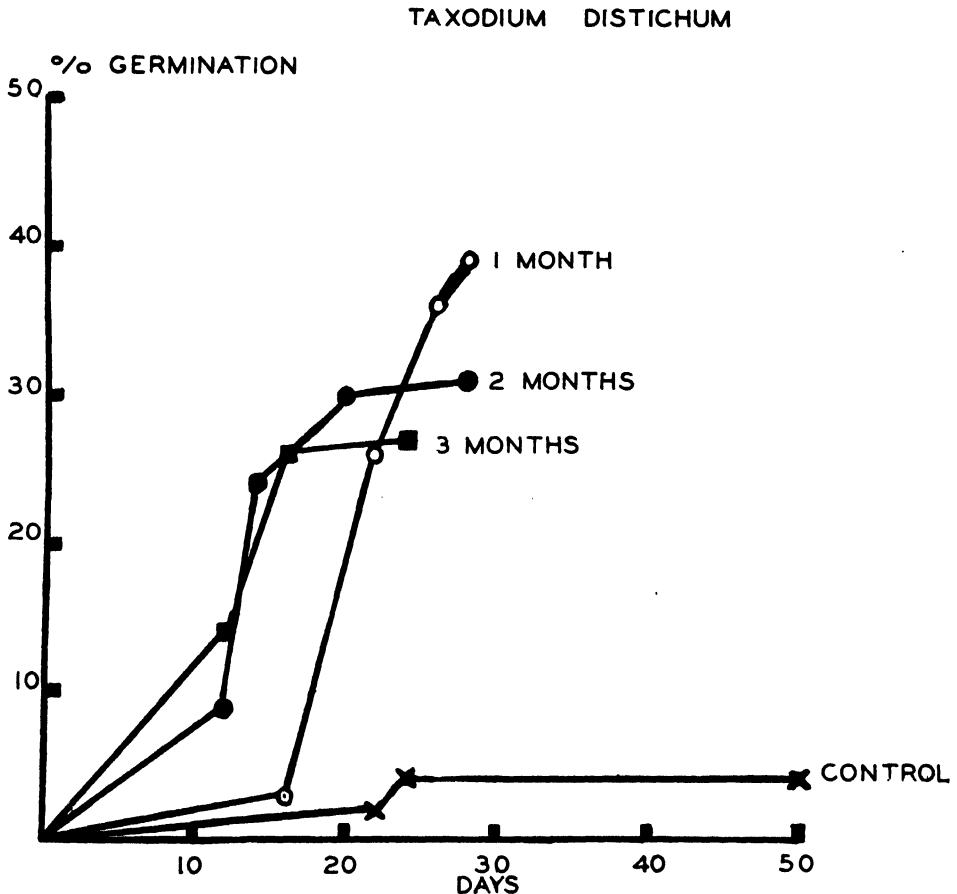
A lot of 1928 seeds of this tree were exceptionally poor in quality. Three hundred seeds were cut open and 88.7 percent of them were empty or molded, only 11.3 percent appearing good.

The usual stratification and sample plantings were made but no seedlings

were obtained. Toumey and Stevens (6) obtained as high as 21 percent germination in 50 days and an average germination of 8.8 percent.

Taxodium distichum

The germination capacity of these seeds as shown by the embryo tests was 76 percent (table 1). Stratification for one month at 5° C. seemed



TEXT FIG. 4. The effect of stratification at 5° C. for one, two, and three months on germination of the seeds of *Taxodium distichum*.

satisfactory for after-ripening the seeds. From seeds thus treated a seedling production of 39 percent was obtained in 28 days. The corresponding untreated seeds germinated to the extent of four percent in the same length of time (table 3 and text fig. 4).

Production of seedlings was much more prompt and complete than that reported by Toumey and Stevens (6). They found that the earliest germination was in 50 days and that in most samples there was no germination within the period of the test. The average germination in 50 days was reported as 0.7 percent and the highest three percent.

Thuya gigantea, *Thuya occidentalis*, and *Thuya orientalis* all responded to two months' stratification at 5° C. In each case the treatment resulted in more prompt and complete stands of seedlings than in the control lots of seeds although this difference was more marked in the case of *Thuya gigantea* (table 3 and text fig. 1, left).

The results agree in general with the germination of *Thuya occidentalis* as reported by Toumey and Stevens (6). They found that the highest germination in 50 days was 65 percent while the average was 33.9 percent. In their samples germination started from ten to 15 days after beginning the test and the crest was usually reached within a period of 25 days.

SUMMARY

1. Experiments on the seeds of several coniferous trees revealed a general favorable effect of low temperature stratification on seed germination.

2. Sample plantings made in the greenhouse after stratification in moist acid peat at 5° C. for a period of two months not only saves time in seedling production but also produces more complete seedling stands in the majority of cases. In some instances other stratification temperatures or periods or both prove more advantageous.

3. *Pinus austriaca*, *Pinus Banksiana*, *Pinus Laricio*, and *Pinus ponderosa* gave a much more prompt production of seedlings (stand complete 8 to 48 days sooner) after stratification for two months at 5° C. However, the actual number of seedlings produced from treated and untreated seeds was about the same.

4. *Pinus contorta*, *Pinus contorta Murrayana*, *Pinus Coulteri*, *Pinus densiflora*, *Pinus flexilis*, *Pinus insignis*, *Pinus monticola*, *Pinus resinosa*, *Pinus rigida*, *Pinus Strobus*, *Pinus Thunbergii*, *Abies arizonica*, *Libocedrus decurrens*, *Picea Omorika*, *Picea sitchensis*, *Thuya gigantea*, *Thuya occidentalis*, and *Thuya orientalis* show decided beneficial effects of stratification at 5° C. for a period of two months. Not only are the seedlings produced in a shorter period of time, but the actual number of seedlings produced is greater than in the corresponding controls.

5. Three months' stratification at 10° C. resulted in the best germination (8 percent after 25 days) of *Pinus excelsa* obtained in these tests. This was better than the average control (2 percent in 60 days), but further study is needed.

6. *Pinus koraiensis* responded equally well to stratification for three months at 5° C. or for one or two months at 10° C. However, the best germination (33 percent in 43 days) was obtained after the seeds had been stratified for five months at either 0° or 5° C.

7. *Pinus Lambertiana* seeds gave best germination after stratification for three months at 10° C. *Pinus Lambertiana* H 1928 thus treated yielded 92 percent germination in 25 days while the average control of the same lot had produced no seedlings in 70 days.

8. Stratification of *Cupressus macrocarpa* for two months at 0° C. gave about four times as many seedlings in one-half the time required by untreated seeds.

9. *Picea canadensis* and *Picea excelsa* preferred treatment at 0° C. for two months, after which they attained germinations of 96 and 90 percent, respectively, in 25 days whereas the average controls gave 48 and 53 percents in 26 days. *Picea pungens*, on the other hand, responded equally well to low-temperature treatment at either 0° or 5° C. for one month, germination tests from these conditions showing percentages of 79 and 80 in 16 days with the corresponding check yielding 47 percent in 50 days.

10. *Sciadopitys verticillata* germinates well after three and one-half months in a warm greenhouse (65° to 70° F.). Sample plantings after two, three, four, five, and six months of stratification at constant temperatures of 0°, 5°, 10°, and 15° C. as well as daily and weekly alternating temperatures of 5° to 10° C. failed to show any beneficial effects. Outside plantings in mulched, board-covered, and open soil showed no advantage.

11. One month at 5° C. proved most satisfactory for pretreatment of *Taxodium distichum* seeds. Treated seeds germinated to the extent of 39 percent in 28 days while the untreated seeds gave four percent germination in the same length of time.

12. *Pinus Cembra* and *Sequoia sempervirens* seeds used in this study produced no seedlings at all. Seeds of the former appeared wrinkled and rather dried when the outer coat was removed. Cutting tests of 300 seeds of the latter showed 11.3 percent good embryos.

13. Weekly alternating temperatures of -5° to 5° C. were tried in the case of *Pinus excelsa*, *Pinus koraiensis*, *Pinus Lambertiana*, *Pinus Strobus*, and *Pinus Thunbergii*. This alternate freezing and thawing apparently has no beneficial effect.

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TABLE 2. Final Germination Percentages after Various Time Periods Following Stratification for One, Two, and Three Months at Different Temperatures *

| Species | Months of Stratification | 0° C. | | | 5° C. | | | 10° C. | | | Average Control | | |
|--|--------------------------|-------------------|--------------|------|-------------------|--------------|------|-------------------|--------------|------|-------------------|--------------|------|
| | | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days |
| <i>Abies arizonica</i> , 1928..... | 1 | 43 | 62 | 41 | 25 | 36 | 17 | 0 | 0 | 50 | | | |
| | 2 | 28 | 41 | 14 | 36 | 52 | 14 | — | — | — | 2 | 3 | 18 |
| | 3 | 27 | 39 | 18 | 12 | 17 | 18 | 22 | 32 | 18 | | | |
| <i>Cupressus macrocarpa</i> , 1928..... | 1 | 19 | 32 | 18 | 16 | 27 | 14 | 13 | 22 | 18 | | | |
| | 2 | 22 | 37 | 23 | 11 | 19 | 18 | 14 | 24 | 18 | 6 | 10 | 50 |
| | 3 | 15 | 25 | 9 | 13 | 22 | 12 | — | — | — | | | |
| <i>Libocedrus decurrens</i> , 30 seeds each, 1928..... | 1 | 23 | 58 | 24 | 13 | 33 | 17 | 7 | 18 | 24 | | | |
| | 2 | 20 | 50 | 15 | 20 | 50 | 15 | 3 | 8 | 11 | 1 | 3 | 50 |
| <i>Picea canadensis</i> , 1928..... | 1 | 85 | 115 | 19 | 48 | 65 | 19 | — | — | — | | | |
| | 2 | 96 | 130 | 25 | 29 | 39 | 25 | — | — | — | 48 | 65 | 26 |
| <i>Picea excelsa</i> , 1928..... | 1 | 66 | 69 | 19 | 65 | 68 | 26 | — | — | — | | | |
| | 2 | 90 | 94 | 25 | 69 | 72 | 25 | — | — | — | 53 | 55 | 26 |
| <i>Picea Omorika</i> , 1928..... | 1 | 58 | 72 | 22 | 58 | 72 | 14 | 54 | 67 | 22 | | | |
| | 2 | 58 | 72 | 15 | 58 | 72 | 15 | 66 | 81 | 15 | 10 | 12 | 16 |
| <i>Picea pungens</i> , 1928..... | 1½ | 79 | 85 | 16 | 80 | 86 | 16 | 74 | 80 | 16 | | | |
| | 2 | 71 | 76 | 17 | 50 | 54 | 17 | 79 | 85 | 17 | 39 | 42 | 20 |
| | 3 | 40 | 43 | 44 | 38 | 41 | 44 | 32 | 34 | 44 | | | |
| <i>Picea sitchensis</i> , 1928..... | 1 | 29 | 37 | 28 | 16 | 20 | 34 | 20 | 25 | 34 | | | |
| | 2 | 10 | 13 | 27 | 24 | 30 | 27 | 32 | 41 | 27 | 4 | 5 | 40 |
| | 3 | 18 | 23 | 25 | 12 | 15 | 20 | 7 | 9 | 25 | | | |
| <i>Pinus austriaca</i> , 1927..... | 1 | 9 | 9 | 15 | 5 | 5 | 15 | 7 | 7 | 15 | | | |
| | 2 | 4 | 4 | 14 | 16 | 16 | 14 | — | — | — | 20 | 20 | 50 |
| | 3 | 10 | 10 | 25 | 9 | 9 | 30 | — | — | — | | | |
| <i>Pinus austriaca</i> , 1928..... | 1 | 49 | 52 | 18 | 50 | 53 | 18 | 27 | 28 | 14 | | | |
| | 2 | 46 | 48 | 18 | 50 | 53 | 18 | — | — | — | 46 | 48 | 18 |
| | 3 | 49 | 52 | 12 | 41 | 43 | 12 | — | — | — | | | |
| <i>Pinus Banksiana</i> , 1927..... | 1 | 43 | 44 | 10 | 43 | 44 | 10 | — | — | — | | | |
| | 2 | 64 | 66 | 14 | 62 | 64 | 21 | — | — | — | 57 | 59 | 26 |
| | 3 | 48 | 50 | 37 | 57 | 59 | 30 | — | — | — | | | |
| <i>Pinus Banksiana</i> , 1928..... | 1 | 56 | 62 | 11 | 54 | 60 | 18 | 71 | 79 | 18 | | | |
| | 2 | 40 | 44 | 18 | 61 | 68 | 18 | 66 | 73 | 18 | 45 | 50 | 18 |
| | 3 | 58 | 64 | 12 | 58 | 64 | 12 | — | — | — | | | |

* These percentages are not absolute finals but they represent the "crest" of the germination in each case.

TABLE 2.—Continued

| Species | Months of Stratification | 0° C. | | | 5° C. | | | 10° C. | | | Average Control | | |
|---|--------------------------|-------------------|--------------|------|-------------------|--------------|------|-------------------|--------------|------|-------------------|--------------|------|
| | | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days |
| <i>Pinus contorta</i> , 1928..... | 1 | 69 | 70 | 14 | 77 | 78 | 11 | 57 | 58 | 45 | | | |
| | 2 | 67 | 68 | 18 | 86 | 89 | 18 | 65 | 66 | 23 | 47 | 47 | 18 |
| | 3 | 64 | 65 | 12 | 84 | 85 | 14 | — | — | — | | | |
| <i>Pinus contorta</i> Murrayana, 1927..... | 1 | 1 | 1 | 15 | 2 | 2 | 15 | — | — | — | | | |
| | 2 | 2 | 2 | 11 | 6 | 6 | 8 | — | — | — | 4 | 4 | 16 |
| | 3 | 0 | 0 | 50 | 0 | 0 | 50 | 6 | 6 | 30 | | | |
| <i>Pinus contorta</i> Murrayana, I, 1928..... | 1 | 80 | 82 | 13 | 83 | 85 | 13 | 71 | 72 | 45 | | | |
| | 2 | 77 | 79 | 14 | 90 | 92 | 14 | 63 | 64 | 23 | 63 | 64 | 24 |
| | 3 | 72 | 73 | 9 | 87 | 89 | 12 | 53 | 53 | 20 | | | |
| <i>Pinus contorta</i> Murrayana, II, 1928..... | 1 | 70 | 71 | 14 | 81 | 82 | 14 | 31 | 31 | 45 | | | |
| | 2 | 59 | 60 | 14 | 64 | 65 | 11 | 27 | 27 | 27 | 40 | 40 | 20 |
| | 3 | 56 | 57 | 12 | 72 | 73 | 9 | 44 | 44 | 15 | | | |
| <i>Pinus contorta</i> Murrayana, III, 1928..... | 1 | 75 | 77 | 26 | 77 | 79 | 26 | 59 | 60 | 32 | | | |
| | 2 | 79 | 81 | 18 | 78 | 80 | 18 | 18 | 18 | 27 | 28 | 29 | 40 |
| | 3 | 82 | 84 | 9 | 94 | 96 | 15 | 18 | 18 | 25 | | | |
| <i>Pinus Coulteri</i> , 1928..... | 1 | 80 | 83 | 24 | 90 | 94 | 19 | 70 | 73 | 24 | | | |
| | 2 | 100 | 104 | 19 | 100 | 104 | 19 | 100 | 104 | 19 | 0 | 0 | 70 |
| | 3 | 100 | 104 | 25 | 88 | 92 | 25 | 100 | 104 | 25 | | | |
| <i>Pinus densiflora</i> , 1927..... | 1 | 57 | 59 | 31 | 68 | 71 | 25 | — | — | — | | | |
| | 2 | 76 | 79 | 21 | 70 | 73 | 24 | — | — | — | 38 | 40 | 40 |
| | 3 | 36 | 38 | 30 | 68 | 71 | 30 | — | — | — | | | |
| <i>Pinus densiflora</i> , 1928..... | 1 | 63 | 67 | 23 | 60 | 64 | 23 | 82 | 87 | 23 | | | |
| | 2 | 66 | 70 | 23 | 52 | 55 | 23 | — | — | — | 45 | 48 | 28 |
| | 3 | 57 | 61 | 12 | 67 | 71 | 20 | — | — | — | | | |
| <i>Pinus excelsa</i> , 1927..... | 1 | 0 | 0 | 60 | 0 | 0 | 60 | 2 | 3 | 28 | | | |
| | 2 | 0 | 0 | 60 | 0 | 0 | 60 | 2 | 3 | 21 | 2 | 3 | 60 |
| | 3 | 0 | 0 | 60 | 1 | 1 | 37 | 2 | 3 | 25 | | | |
| <i>Pinus excelsa</i> , 1928..... | 1 | 2 | 3 | 34 | 1 | 1 | 18 | 0 | 0 | 45 | | | |
| | 2 | 0 | 0 | 49 | 1 | 1 | 27 | 1 | 1 | 14 | 1 | 1 | 12 |
| | 3 | 0 | 0 | 53 | 2 | 3 | 20 | 8 | 11 | 25 | | | |
| <i>Pinus flexilis</i> , 1927..... | 1 | 86 | 104 | 17 | 94 | 113 | 17 | 42 | 51 | 22 | | | |
| | 2 | 86 | 104 | 14 | 100 | 120 | 18 | 80 | 96 | 23 | 46 | 55 | 28 |
| | 3 | 96 | 116 | 12 | 88 | 106 | 9 | 96 | 116 | 15 | | | |
| <i>Pinus flexilis</i> , 1928..... | 1 | 45 | 83 | 11 | 35 | 65 | 14 | 55 | 102 | 23 | | | |
| | 2 | 18 | 33 | 23 | 40 | 74 | 23 | 32 | 59 | 23 | 45 | 83 | 28 |
| | 3 | 36 | 67 | 15 | 36 | 67 | 15 | 48 | 89 | 25 | | | |

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TABLE 2.—Continued

| Species | Months of Stratification | 0° C. | | | 5° C. | | | 10° C. | | | Average Control | | |
|-------------------------------------|--------------------------|-------------------|--------------|------|-------------------|--------------|------|-------------------|--------------|------|-------------------|--------------|------|
| | | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days |
| <i>Pinus insignis</i> , 1927 | 1 | 69 | 71 | 33 | 81 | 84 | 18 | 28 | 29 | 15 | 62 | 64 | 50 |
| | 2 | 75 | 78 | 24 | 75 | 78 | 14 | — | — | — | — | — | — |
| | 3 | 62 | 64 | 30 | 84 | 87 | 30 | 86 | 89 | 25 | — | — | — |
| <i>Pinus insignis</i> , 1928 | 1 | 74 | 80 | 28 | 77 | 83 | 28 | 85 | 91 | 28 | 65 | 70 | 28 |
| | 2 | 62 | 67 | 14 | 79 | 85 | 18 | 76 | 82 | 18 | — | — | — |
| <i>Pinus koraiensis</i> , I, 1928 | 3 | 60 | 65 | 15 | 84 | 90 | 20 | 90 | 97 | 15 | — | — | — |
| | 1 | 0 | — | 60 | 15 | — | 45 | — | — | — | — | — | — |
| | 2 | 8 | — | — | 8 | — | 27 | 8 | — | 82 | 0 | 0 | 70 |
| <i>Pinus koraiensis</i> , II, 1928 | 3 | 0 | — | 53 | 20 | — | 25 | 0 | — | 53 | — | — | — |
| | 2 | 0 | 0 | 47 | 0 | 0 | 47 | 10 | 10 | 28 | 0 | 0 | 70 |
| <i>Pinus Lambertiana</i> , 1927 | 3 | 0 | 0 | 47 | 0 | 0 | 47 | 10 | 10 | 28 | — | — | — |
| | 1 | 10 | 10 | 25 | 10 | 10 | 25 | 0 | 0 | 70 | 2 | 2 | 70 |
| | 2 | 14 | 14 | 62 | 33 | 33 | 62 | 9 | 9 | 55 | — | — | — |
| | 3 | 72 | 72 | 30 | 67 | 67 | 37 | 47 | 47 | 37 | — | — | — |
| <i>Pinus Lambertiana</i> , I, 1928 | 1 | 0 | 0 | 60 | 0 | 0 | 60 | 0 | 0 | 60 | 8 | 9 | 70 |
| | 2 | 28 | 32 | 64 | 32 | 36 | 64 | 8 | 9 | 55 | — | — | — |
| <i>Pinus Lambertiana</i> , II, 1928 | 3 | 30 | 34 | 20 | 50 | 57 | 35 | 30 | 34 | 25 | — | — | — |
| | 2 | 80 | 103 | 19 | 40 | 51 | 22 | 28 | 36 | 22 | 0 | 0 | 70 |
| <i>Pinus Laricio</i> , 1927 | 3 | 72 | 92 | 25 | 64 | 85 | 25 | 92 | 118 | 25 | — | — | — |
| | 1 | 42 | 48 | 15 | 59 | 66 | 15 | — | — | — | 56 | 64 | 26 |
| | 2 | 48 | 55 | 14 | 50 | 57 | 14 | — | — | — | — | — | — |
| <i>Pinus Laricio</i> , 1928 | 3 | 34 | 39 | 22 | 33 | 38 | 25 | — | — | — | — | — | — |
| | 1 | 32 | 32 | 13 | 21 | 21 | 7 | 6 | 6 | 13 | 11 | 11 | 26 |
| <i>Pinus monticola</i> , 1927 | 2 | 7 | 7 | 12 | 13 | 13 | 6 | 1 | 1 | 12 | — | — | — |
| | 1 | 20 | 25 | 33 | 10 | 13 | 28 | 23 | 29 | 28 | 12 | 15 | 60 |
| | 2 | 37 | 47 | 62 | 26 | 34 | 62 | — | — | — | — | — | — |
| <i>Pinus monticola</i> , 1928 | 3 | 24 | 30 | 37 | 41 | 54 | 37 | 22 | 28 | 50 | — | — | — |
| | 2 | 28 | 29 | 47 | 15 | 16 | 21 | 7 | 7 | 47 | 10 | 11 | 50 |
| <i>Pinus ponderosa</i> , I, 1927 | 3 | 30 | 32 | 23 | 18 | 19 | 16 | 17 | 18 | 28 | — | — | — |
| | 1 | 74 | 85 | 15 | 76 | 87 | 10 | 78 | 90 | 15 | 73 | 84 | 22 |
| | 2 | 78 | 90 | 14 | 70 | 80 | 17 | 68 | 78 | 14 | — | — | — |
| <i>Pinus ponderosa</i> , II, 1927 | 3 | — | — | — | 63 | 72 | 37 | — | — | — | — | — | — |
| | 1 | 97 | 103 | 28 | 97 | 103 | 28 | 88 | 94 | 31 | 79 | 84 | 40 |
| | 2 | 60 | 64 | 22 | 71 | 76 | 14 | 46 | 49 | 22 | — | — | — |

| Species | Months of Stratification | 0° C. | | | 5° C. | | | 10° C. | | | Average Control | | |
|---|--------------------------|-------------------|--------------|------|-------------------|--------------|------|-------------------|--------------|------|-------------------|--------------|------|
| | | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days | Appar-ent % Germ. | Real % Germ. | Days |
| <i>Pinus ponderosa</i> , 1928..... | 1 | 47 | 55 | 14 | 36 | 42 | 14 | 62 | 73 | 18 | 48 | 56 | 16 |
| | 2 | 30 | 35 | 18 | 47 | 55 | 14 | 39 | 46 | 11 | | | |
| | 3 | 15 | 18 | 12 | 18 | 21 | 12 | | | | | | |
| <i>Pinus resinosa</i> , 1927..... | 1 | 88 | 91 | 18 | 83 | 86 | 18 | 84 | 87 | 15 | 64 | 66 | 24 |
| | 2 | 73 | 75 | 14 | 91 | 94 | 17 | 83 | 86 | 17 | | | |
| | 3 | 76 | 78 | 22 | 95 | 98 | 22 | 89 | 92 | 25 | | | |
| <i>Pinus resinosa</i> , 1928..... | 1 | 89 | 94 | 18 | 84 | 88 | 18 | 91 | 96 | 18 | 67 | 72 | 24 |
| | 2 | | | | 89 | 94 | 18 | 97 | 102 | 23 | | | |
| | 3 | 90 | 95 | 15 | 85 | 89 | 15 | 79 | 83 | 15 | | | |
| <i>Pinus rigida</i> , 1928..... | 1 | 86 | 95 | 18 | 95 | 104 | 18 | 79 | 87 | 23 | 32 | 35 | 40 |
| | 2 | 79 | 87 | 14 | 92 | 101 | 14 | 96 | 105 | 14 | | | |
| | 3 | 83 | 91 | 12 | 88 | 97 | 12 | 85 | 93 | 15 | | | |
| <i>Pinus Strobus</i> , 1927..... | 1 | 57 | 59 | 33 | 47 | 49 | 31 | 59 | 61 | 31 | 3 | 3 | 60 |
| | 2 | 70 | 73 | 41 | 71 | 74 | 41 | 76 | 79 | 41 | | | |
| | 3 | 56 | 58 | 37 | 64 | 67 | 37 | 52 | 54 | 40 | | | |
| <i>Pinus Strobus</i> , 1928..... | 1 | 27 | 28 | 45 | 26 | 27 | 28 | 16 | 16 | 45 | 6 | 6 | 40 |
| | 2 | 35 | 36 | 23 | 26 | 27 | 23 | 46 | 47 | 23 | | | |
| | 3 | 28 | 29 | 35 | 33 | 34 | 25 | 16 | 16 | 25 | | | |
| <i>Pinus Thunbergii</i> , 1927..... | 1 | 12 | 14 | 25 | 16 | 19 | 25 | 15 | 18 | 15 | 8 | 9 | 30 |
| | 2 | 10 | 12 | 21 | 20 | 24 | 21 | 8 | 9 | 14 | | | |
| | 3 | 7 | 8 | 40 | 6 | 7 | 37 | 6 | 7 | 25 | | | |
| <i>Pinus Thunbergii</i> , 1928..... | 1 | 14 | 18 | 23 | 18 | 23 | 28 | 7 | 9 | 18 | | | |
| | 2 | 7 | 9 | 23 | 11 | 14 | 23 | 12 | 15 | 23 | 10 | 13 | 28 |
| | 3 | 10 | 13 | 25 | 5 | 6 | 12 | 6 | 8 | 15 | | | |
| <i>Sciadopitys verticillata</i> , 1928..... | 2 | 14 | 16 | 148 | 20 | 22 | 101 | 50 | 56 | 101 | | | |
| | 3 | 42 | 49 | 117 | 42 | 49 | 117 | 36 | 40 | 117 | 28 | 31 | 100 |
| <i>Taxodium distichum</i> , 1928..... | 1 | 19 | 25 | 28 | 39 | 51 | 28 | 40 | 53 | 28 | | | |
| | 2 | 32 | 42 | 37 | 31 | 41 | 27 | 50 | 66 | 27 | 4 | 5 | 24 |
| | 3 | 43 | 57 | 28 | 27 | 36 | 23 | 33 | 43 | 23 | | | |
| <i>Thuja gigantea</i> , 1928..... | 1 | 72 | 90 | 16 | 67 | 84 | 16 | 56 | 70 | 19 | | | |
| | 2 | 43 | 54 | 12 | 57 | 71 | 14 | 43 | 54 | 14 | 10 | 13 | 24 |
| | 3 | 60 | 75 | 14 | 74 | 93 | 14 | 56 | 70 | 14 | | | |
| <i>Thuja occidentalis</i> , 1928..... | 1 | 28 | 51 | 18 | 50 | 91 | 24 | 41 | 75 | 30 | 24 | 44 | 24 |
| | 2 | 40 | 73 | 20 | 33 | 60 | 27 | 44 | 80 | 20 | | | |
| | 3 | 38 | 69 | 16 | 22 | 40 | 16 | 40 | 73 | 16 | | | |
| <i>Thuja orientalis</i> , 1928..... | 1 | 20 | 27 | 18 | 21 | 28 | 18 | 26 | 35 | 18 | 26 | 35 | 24 |
| | 2 | 33 | 45 | 27 | 33 | 45 | 18 | 9 | 12 | 14 | | | |
| | 3 | 36 | 49 | 12 | 30 | 41 | 12 | | | | | | |

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TABLE 3.—Continued

| Species | Months of Stratification | Number of Days | | | | | | | | | | | | | | | | | | |
|---|--------------------------|-------------------------|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | 40 | 50 | 60 | 70 |
| | | Germination Percentages | | | | | | | | | | | | | | | | | | |
| <i>Pinus flexilis</i> , 25 seeds each, 1928..... | Control | | | | | | 3 | | 9 | 12 | 21 | 24 | 32 | 36 | 46 | | 50 | | | |
| | 1 | | | | | | 10 | 35 | | | | | | | | | | | | |
| | 2 | | | | | | | 32 | | 36 | | | 40 | | | | | | | |
| | 3 | | | | | | 28 | | 36 | | | | | | | | | | 44 | |
| <i>Pinus insignis</i> , 1927..... | Control | | | | | | 9 | 21 | 23 | 34 | 40 | | | 42 | 45 | | | | | |
| | 1 | | | | | 64 | | | 80 | 81 | | | | | | | | | | |
| | 2 | | | | | | 54 | 75 | | | | | | | | | | | | |
| | 3 | | | | | | | 74 | | | 82 | | | | | 84 | | | | |
| <i>Pinus insignis</i> , 1928..... | Control | | | | | | | | 9 | 16 | | 33 | 36 | 46 | 49 | 53 | 54 | 62 | 67 | 69 |
| | 1 | | | | | | 5 | 60 | | 71 | | | 75 | | 77 | | 78 | | | |
| | 2 | | | | | | 52 | 64 | | 79 | | | | | | | | | | |
| | 3 | | | | | 43 | 77 | | 80 | | 84 | | | | | | | | | |
| <i>Pinus koraiensis</i> , 1, 25 seeds each, 1928..... | Control | | | | | | 1 | | 6 | 25 | 38 | | 56 | 58 | 65 | | 68 | 69 | | |
| | 1 | | | | | | | | | | | | | | | | 10 | 15 | | |
| | 2 | | | | | | | | | | | | | | | 8 | | | | |
| | 3 | | | | | | | | | | | | | 20 | | | | | | |
| <i>Pinus Lambertiana</i> , 25 seeds each, 1927..... | Control | | | | | | | | | | | | | | | | | | 0 | |
| | 1 | | | | | | | | 7 | | | | | 10 | | | | | | |
| | 2 | | | | | | | | | 11 | | | 18 | | | 19 | | 29 | 33 | |
| | 3 | | | | | | | | 5 | | 17 | 40 | | 56 | | | 67 | | | |
| <i>Pinus Lambertiana</i> , 1, 25 seeds each, 1928..... | Control | | | | | | | | | | | | | | | | | 1 | 2 | |
| | 2 | | | | | | | | | | | | 8 | | | | 16 | | 32 | |
| | 3 | | | | | | 10 | | 20 | | | | | 30 | | | 50 | | | |
| | Control | | | | | | | | | | | | | | | | | | 8 | |
| <i>Pinus Lambertiana</i> , 11, 25 seeds each, 1928..... | 2 | | | | | | | | | | | | | | | | | | | |
| | 3 | | | | | | | | | | | | | | | | | | | |
| | Control | | | | | | | | | | | | | | | | | | | |
| | 2 | | | | | | | 4 | | | 32 | 40 | | | | | | | | |
| <i>Pinus Laricio</i> , 1927..... | Control | | | | | | | | | | | | | | | | | | 0 | |
| | 1 | | | | 44 | 55 | | | 59 | | | | | | | | | | | |
| | 2 | | | | 49 | 50 | | | | | | | | | | | | | | |
| | 3 | | | 24 | | | | | | | | | | | | | | | | |
| | Control | | | | | 11 | 17 | 22 | 32 | 35 | 46 | 51 | 53 | 56 | | 57 | | 58 | | |

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TABLE 3.—Continued

| Species | Months of Stratification | Number of Days | | | | | | | | | | | | | | | | | | |
|--|--------------------------|-------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| | | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 | 18 | 20 | 22 | 24 | 26 | 28 | 30 | 40 | 50 | 60 | 70 |
| | | Germination Percentages | | | | | | | | | | | | | | | | | | |
| <i>Pinus Laricio</i> , 1928..... | 1 | | | 21 | | | | | | | | | | | | | | | | |
| | 2 | | 13 | | | | | | | | | | | | | | | | | |
| | 3 | | | | 31 | | | | | | | | | | | | | | | |
| | Control | | | | 1 | | | | 5 | | | | | | 11 | | | | | |
| <i>Pinus monticola</i> , 1927..... | 1 | | | | | | | | 7 | 8 | | 9 | | | 10 | | | | | |
| | 2 | | | | | 4 | | 6 | | 10 | | 16 | | | | 18 | 20 | | 25 | 26 |
| | 3 | | | | | | | | 8 | | 12 | 20 | | | 23 | | 41 | | 43 | 48 |
| | Control | | | | | | | | | | | 1 | | | | 2 | 4 | 8 | 11 | 12 |
| <i>Pinus monticola</i> , 1928..... | 1 | | | | | | | | | | | 15 | | | | | | | | |
| | 2 | | | 2 | | | 1 | | 18 | | | | | | | | | | | |
| | Control | | | | | | | | | | | | | | | 2 | | 9 | 10 | |
| <i>Pinus ponderosa</i> , I, 1927..... | 1 | | | 52 | 76 | | | | | | | | | | | | | | | |
| | 2 | | | 21 | | 69 | | | | 70 | | | | | | | | | | |
| | 3 | | | | | | | | 58 | 62 | | | | | | | | 63 | | |
| | Control | | | | 20 | 34 | 40 | 47 | | | 69 | 73 | | 74 | | | | | | |
| <i>Pinus ponderosa</i> , II, 1927..... | 1 | | | | | | | | | | | | | | | 97 | | | | |
| | 2 | | | | 69 | | 71 | | | | | | | | | | | | | |
| | Control | | | | 36 | | 43 | | | | | 44 | | | | 64 | | 79 | | |
| <i>Pinus ponderosa</i> , 1928..... | 1 | | | | | 34 | 36 | | | | | | | | | | | 37 | 38 | |
| | 2 | | | 33 | | 45 | | | | 47 | | | | | | | | | | |
| | 3 | | | | 28 | 36 | | | | | | | | | | | | | | |
| | Control | | | | 3 | 33 | 47 | 48 | | | | | | | 50 | | | | | |
| <i>Pinus resinosa</i> , 1927..... | 1 | | | | 70 | | | | 82 | 83 | | | | | | | | | | |
| | 2 | | | | | 30 | 88 | | | 91 | | | | | | | | | | |
| | 3 | | | | | | | | 82 | | 89 | 95 | | | | | | | | |
| | Control | | | | | | | 25 | 42 | | | 52 | 64 | | | | | | | |
| <i>Pinus resinosa</i> , 1928..... | 1 | | | | | 65 | 82 | | | 84 | | | | | | | | | 85 | |
| | 2 | | | | | 38 | | | | 89 | | | | | | | | | | |
| | 3 | | | | 14 | 72 | | | 85 | | | | | | | | | | | |
| | Control | | | | | | 12 | 31 | 62 | 65 | | | 67 | | | | | | 68 | |
| <i>Pinus rigida</i> , 1928..... | 1 | | | | | 87 | 90 | | | 95 | | | 96 | | | | | 99 | | |
| | 2 | | | | | 87 | 92 | | | | | | | | | | | | | |
| | 3 | | | 29 | 84 | 88 | | | 89 | | | | | | | | | | | |
| | Control | | | | | | 3 | 7 | 10 | 15 | 18 | | 21 | | 25 | | | 32 | 33 | |

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A MICROCHEMICAL STUDY OF HEMICELLULOSES OF ENDOSPERMS AND COTYLEDONS¹

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HISTORICAL

Hemicelluloses occur in several plant tissues, particularly in wood, seed coats, and endosperms, and in fungi. Very little is known about their exact character as they occur in cell walls. Their presence is determined macrochemically by the products of their hydrolysis with weak acids. A review of the literature shows a considerable variation in properties of hemicelluloses obtained from different sources. Consequently definitions for the hemicelluloses as a distinct class of compounds do not entirely agree. Differences of opinion result from the number of hemicelluloses studied and from the methods of study used. The macrochemical methods consist of hydrolyzing the tissues with dilute acids or, more commonly, of extracting tissue with one to five percent alkali and testing the alcoholic precipitate from the extract. This precipitate is called hemicellulose and its properties are considered to be identical with those of hemicellulose regardless of the fact that the tissue from which the extract was obtained may contain non-hemicellulose compounds such as pectic compounds, soluble in dilute alkali and capable of precipitation by alcohol. The main difficulty, however, of arriving at an acceptable definition of the hemicelluloses as a group lies in the heterogeneity of the various hemicellulose substances. An account of the opinions of the more prominent workers will demonstrate the diversity of the hemicelluloses as well as the scarcity of knowledge concerning them.

Schulze and Tollens (33) worked with thickened walls both microchemically and macrochemically. They suggested that the term hemicellulose be given to cell wall substances which differ from cellulose in their non-resistance to dilute acids or alkalis but are like cellulose in that they

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give a positive hydrocellulose reaction, are soluble in copper oxid-ammonia, and are resistant to Schulze's reagent (nitric acid and potassium chlorate). Gilson (13) considered carbohydrate substances of membranes which did not color blue with chlorzinc iodid as hemicelluloses. Grafe's concept of hemicelluloses is essentially that of Schulze but he adds that in contrast to cellulose, some hemicelluloses give a blue color in a weak iodine solution as does starch. Grafe gives dextrose, mannose, galactose, xylose, and arabinose as the products of hydrolysis of hemicellulose and states that hemicellulose dissolves in glycerine at 300° C. Tunmann (35) includes all unsuberized, non-lignified walls which are hydrolyzed by treatment with dilute acids as hemicelluloses. Molisch (20) says they are those substances which are easily dissolved in hot dilute acids, as 1 percent HCl, which give the hydrocellulose reaction, or become colored directly blue with iodine. Schorger (28) says that a hemicellulose may be broadly defined as a polysaccharid soluble in dilute alkalies and convertible into simple sugars by heating with dilute acids at atmospheric pressure. In contrast to starch and several other substances it is insoluble in hot water. Van Wisselingh (37) describes hemicelluloses as various carbohydrates contained in cell walls which yield mannose, galactose, arabinose, and xylose upon hydrolysis.

Another group of workers considered hemicelluloses pectic in nature. Czapek (9) considered classing the pectic substances as hemicelluloses. Substances determined as pectic compounds by Mangin (19) have been called hemicelluloses by others. O'Dwyer (22), in making a study of the hemicelluloses which she isolated from wood of white oak and American elm by extracting with 4 percent alkali, detected a certain amount of galacturonic acid. As galacturonic acid is a compound which has been associated previously with the pectic substances, O'Dwyer considered her detection a proof that the hemicelluloses are related to the pectic substances. Since some pectic compounds are soluble in dilute alkali, the galacturonic acid reported may be from pectic materials rather than from hemicelluloses.

There are several summaries of the literature on hemicelluloses. Grafe (14) deals with the work up to 1911, and has attempted to organize the heterogeneous collection of facts by making a classification of the hemicelluloses. He discusses his groups separately as amylane, mannan, galactomannan, glycomannan, fructomannan, mannocellulose, galactan, α , β , γ , and δ galactans, paragalactan, galactoaraban, galacto-xylan, galactit, amyloid, galactogen, levulomannan, dextran, and para-dextran. Under each group he cites the plant tissue in which the substance may be found, how it has been isolated, and the author. He gives the hydrolytic products from which the various names are derived and some of the solubilities and color reactions of the isolated substances. Czapek (9) briefly reviews the work up to 1913. He cites some of the work on pentosans and methyl pentosans. Van Wisselingh (37) gives a brief summary of work on hemi-

celluloses up to 1924. He names tissues that have been studied and describes a number of macrochemical tests including the identification of the hydrolytic products by means of osazones.

Sachs in 1862 reported that the thickened walls of the date seed disappeared during germination. Frank (11) in 1866 announced that the thickened walls of *Tropaeolum* cotyledons disappeared during germination. Schellenberg (25), Reiss (24), and Schulze (29) made microscopic examinations of seeds during germination. Schellenberg used the iodine reagents to trace the disappearance of the hemicellulose in seeds of some species of Plantaginaceae. Schulze compared sections of ungerminated seeds of *Lupinus* species boiled in dilute H_2SO_4 with those of germinated seeds untreated and found a similar decrease of galactan in each. In another investigation (30), he determined quantitatively the decrease in galactan by mucic acid tests. Microchemically he proved that the cotyledon walls were just as thick after the first stages of germination as before. He called the remaining material in the wall paragalactan and stated that it gave a cherry red solution with phloroglucin and HCl when heated. Reiss studied the disintegration of the thickened wall during germination by means of Congo red and solubility in copper oxid-ammonia reactions. With some seeds he used iodine reactions.

No distinctive localization reaction has been reported for the hemicelluloses. Potassium iodid solution of iodine was used by Vogel and Schleiden (38) to distinguish the special group of hemicelluloses known as amyloid; but substances which they have isolated and called amyloid do not all become colored in the natural state, as will be shown later. The osazone test used to distinguish between the sugars obtained by hydrolizing cellulose and hemicellulose are not good for localization tests within the walls although they are applicable for different tissues. The osazones form in the neutralized solution of sugar both inside and outside of the cells but not in the walls. The disappearance of certain layers of the walls during hydrolysis may not be relied on as a test. Sections ($10\ \mu$ thick) of endosperms of several seeds were hydrolized by heating eight hours with 3 percent H_2SO_4 . The neutralized solutions yielded copious precipitates of osazones showing that a considerable portion of the material in the walls had been hydrolized, but the cell walls of the sections were not appreciably thinner after the hydrolysis.

PURPOSE OF INVESTIGATION

The object of this investigation was to make a comparative survey of the chemical and crystallographic properties of several hemicelluloses and to discover some properties and methods which would be of value in distinguishing hemicelluloses from other membrane substances microchemically. Endosperms and cotyledons of seeds were used as a source of hemicellulose. Ten seeds were selected as follows:

Endosperms of:

Phoenix dactylifera
Coffea arabica
Strychnos nux-vomica
Iris pallida ²
Diospyros virginiana ²
Asparagus Sprengeri ²

Cotyledons of:

Impatiens balsamina
Lupinus hirsutus
Tropaeolum majus
Primula officinalis

Seeds of *Phoenix dactylifera* and *Coffea arabica* (unroasted) were obtained from a grocery; those of *Strychnos nux-vomica* from a druggist; those of *Impatiens balsamina*, *Lupinus hirsutus*, *Tropaeolum majus*, and *Primula officinalis* from seedsmen; those of *Iris pallida* and *Asparagus Sprengeri* from the garden and greenhouse of the botanical department; and those of *Diospyros virginiana* from Illinois. While the investigation was carried on, all the seeds were kept in dry storage at room temperature.

The literature concerning the individual species will be discussed under their reactions with the various reagents. However, as only a little confirmatory work on the hydrolytic products was completed in this investigation, a list of the sugars found upon hydrolysis by other workers follows (table 1). The various authors used the osazone methods and the mucic acid test for the identification of the sugars.

TABLE 1

| Seed | Hydrolytic Products | Author | Seed | Hydrolytic Products | Author |
|---|---|-----------------------|-----------------------------|------------------------|--------------------|
| <i>Iris germanica</i> <i>Iris pseudacorus</i> <i>Iris foetidissima</i> <i>Iris pallida</i> | mannose arabinose mannose* | Colin et Augem (7) | <i>Strychnos nux-vomica</i> | galactose* mannose | Baker and Pope (3) |
| <i>Asparagus officinalis</i> | mannose fructose glucose galactose | Cake and Bartlett (5) | <i>Lupinus hirsutus</i> | galactose arabinose | Schulze (30) |
| <i>Disopyros Kaki</i> <i>Disopyros virginiana</i> | mannose mannose* | Loew and Ishii (18) | <i>Tropaeolum majus</i> | galactose xylose | Winterstein (39) |
| <i>Phoenix dactylifera</i> | galactose d-glucose d-mannose | Gruss (15) | <i>Primula officinalis</i> | No definite data found | |
| <i>Coffea arabica</i> | galactose* mannose | Schulze (30) | <i>Impatiens balsamina</i> | galactose xylose | Winterstein (39) |

* Reactions for these sugars were obtained in macrochemical analysis in this investigation.

² Endosperm tissues of these genera have been studied but they were not of the same species.

METHODS

Preparation of the Tissue for Microchemical Tests

The seeds were soaked in distilled water from 1 to 3 days to facilitate sectioning. All sections were cut 10 microns thick on the freezing microtome except where otherwise specified. This uniformity of thickness insured more nearly comparable results. In these thin sections very few cells remained uncut. Consequently cell contents were easily removed by washing and reagents could be brought into direct contact with every layer in the cell wall, thus excluding errors arising from differences in permeability.

Previous to some tests chloral hydrate in water (5 : 2) was used as a clearing agent. Poulsen (23) says that chloral hydrate has the same effect upon fats and volatile oils as alcohol and that it dissolves the same saccharine and amylaceous matters as water and causes the swelling of starch grains. He adds that it swells or dissolves protein matters and is, therefore, frequently useful in clearing tissues. When *Iris* endosperm sections are treated with chloral hydrate for several hours, the pectic middle lamella of the walls swells and a Ruthenium red test is clearer than in untreated sections. Also treatment of *Iris* endosperm with chloral hydrate previous to a macrochemical analysis was found not to interfere with the production of sugar from the walls after they had been thoroughly washed with water. In general chloral hydrate used as a previous treatment was found to hasten the reaction of the other reagents.

Terminology Used for the Layers of the Cell Walls

Three layers have been demonstrated in the cell walls of the endosperms or cotyledons of eight of the seeds studied. In the discussion which follows these three layers will be referred to as follows: (1) the primary wall or middle lamella, (2) the thin secondary wall on each side of the middle lamella, and (3) the tertiary wall, a relatively thick layer formed adjacent to the secondary wall during the later stages in the development of the cell (text fig. 1).

That the three layers in the walls of endosperms of *Coffea* and *Strychnos* and of cotyledons of *Lupinus* and *Tropaeolum* each contain different substances or combinations of substances has been shown by Gilson (13). In the cell walls of endosperms of *Asparagus* and *Phoenix* and of cotyledons of *Tropaeolum* a secondary continuous layer with a high interference color can be demonstrated with a polariscope. The primary wall is isotropic, and the discontinuous tertiary wall has a low interference color. If sections of *Iris* and *Diospyros* endosperms dried at 100° C. for one hour are observed under the polariscope while reacting with (1) chloral hydrate in the case of *Iris* and (2) ZnCl_2HCl reagent in the case of *Diospyros*, the bright secondary wall may be demonstrated. The tertiary layer is the one containing the substance described by Frank (11), Schulze and Tollens (33), and Gilson (13) as hemicellulose.

In *Asparagus* endosperms the tertiary layer dissolves in the cellulose solvents used in this study much more rapidly than the secondary. Solubility tests were made on dried sections in order that the decrease in anisotropic material might be observed. By using the ZnCl_2HCl reagent dilute and then gradually increasing the concentration, the reaction does not proceed too fast for observation. Except for the narrow tracing in the cell outline the anisotropic character of the wall is destroyed immediately. In the light field the discontinuous tertiary layer may be watched as it dissolves, but the anisotropic narrow tracing of the secondary wall is still observable on either side of the primary wall after three hours. By using CuO in NH_4OH the same process occurs but the bright narrow tracings are not so distinctly seen. This method of demonstrating the existence of a secondary wall which is very resistant as compared to the hemicellulose tertiary wall was not successful on the other tissues studied.

RESULTS

Microchemical Reactions

Color Reactions

Chlorzinc-iodid. (25 gms. ZnCl_2 : 8 gms. KI : 1.5 gms. I_2 : 8.5 cc. water.) Hemicelluloses give varying reactions with this reagent. Grafe (14) quotes Schulze and Steiger (32) concerning *Lupinus hirsutus* cotyledons, in which the cell walls become violet. Grüss (15) reports that cell walls of *Phoenix* endosperms have a blue color in chlorzinc-iodid. Van Wisselingh found that the tertiary walls of endosperms of *Strychnos* colored blue with chlorzinc-iodid. On the other hand, Gilson (13) speaks of carbohydrate substances occurring with cellulose in the cell walls of endosperms and cotyledons which do not color with chlorzinc-iodid. In this research the tertiary walls of endosperms of *Coffea*, *Phoenix*, and *Strychnos* and cotyledons of *Tropaeolum* gave a violet reaction with chlorzinc-iodid (table 2).

Ambronn (1) and Frey (12) report that pleochroism is characteristic of cellulose and of some other substances stained with chlorzinc-iodid. The tertiary walls which give a color reaction with this reagent were examined for this property. Pleochroism was apparent in the walls of *Lupinus* and *Phoenix*, but in the latter it was not very distinct. In the endosperm of *Coffea* the color reaction appeared to be only on the surface of the wall rather than through the cross section of the wall. The color was very pale in the walls of *Strychnos* and *Nasturtium*. The type of color reaction in the last three species may be responsible for the apparent lack of pleochroism.

Phloroglucin (1 percent in alcohol), or *orcinol* (4 percent in water) followed by concentrated *hydrochloric acid*. Van Wisselingh (37) quotes Bertrand as saying that phloroglucin, or any other phenol such as orcinol, and concentrated HCl heated with hexoses, gives a gold color which rapidly changes to orange and finally appears as a brown precipitate, but heated

with pentoses these reagents give a violet color. Since lignin gives a cherry red reaction with cold phloroglucin and HCl, it would be difficult to get a distinctive reaction for a hemicellulose if lignin were present. Another caution must be observed in testing cell walls of seed tissues. A very thin seed coat such as that of *Tropaeolum* may be rich in the furfural-yielding substance upon which the pentosan reaction depends. These substances should be removed or they may be sufficient in the reaction solution to color the whole section by diffusion. Table 2 shows that the tertiary walls of cotyledons of *Lupinus*, *Tropaeolum*, and endosperms of *Iris*, *Phoenix*, *Coffea*, and *Primula* give phloroglucin and orcinol reactions. These reactions were not entirely in accordance with the sugars obtained upon hydrolysis. Large amounts of sectioned tissue of *Iris* and *Lupinus* are necessary to obtain pentosan reactions. Even when large amounts of sectioned tissue of *Phoenix* and *Coffea* are used the gold hexosan reaction obscures the violet pentosan reaction. However, a trace of violet may sometimes be seen before the gold appears.

Iodine in a water solution of potassium iodid. (0.3 gms. I_2 : 1.6 gms. KI: 100 cc. water.) Vogel and Schleiden (38) determined that the cell walls of cotyledons of *Schottia latifolia*, *S. speciosa*, *Hymenaea courbaril*, *Mucuna urens*, and *Tamarindus indica* became colored directly with I_2KI . They named the substance amyloid. Nadelman (21) adds the names of eight other seeds to the above list. The tertiary walls of cotyledons of *Primula* and *Impatiens* have been reported by Reiss (24) to contain amyloid. Table 2 shows that the color reaction of I_2KI on the walls of these cotyledons in the natural state was brown. The only tertiary wall in the seeds studied that colored blue in the natural state by this reagent is that of cotyledons of *Tropaeolum*.

Iodine in a water solution of potassium iodid and sulfuric acid. Concentrations of 12 percent, 26 percent, 35 percent, 43 percent, 50 percent, 75 percent of H_2SO_4 , sp. gr. 1.86 taken as 100 percent, were made up according to volume. These two reagents applied in the order named give a blue color with celluloses and hemicelluloses according to Schleiden (26). The I_2KI and H_2SO_4 tests made in this investigation determined two things:

1. The comparative effects of these reagents upon hemicellulose and cellulose. The two celluloses used for comparison were that of cotton fiber and that found by Shaw (34) in the walls of the palisade cells of the fruit coats of *Nelumbo lutea*. The cellulose from both sources gave the color reaction with I_2KI and 50 percent H_2SO_4 and with higher concentrations but not with lower concentrations of the acid. The results of the tests on hemicelluloses are shown in table 2. The lowest concentration of H_2SO_4 that would produce the color reaction in the tertiary walls of *Diospyros*, *Phoenix*, *Strychnos*, *Coffea* endosperms, and *Lupinus* cotyledons was 50 percent. All concentrations of the acid produced only a brown coloring in the tertiary walls of *Iris* and *Asparagus* endosperms. The tertiary walls

of *Tropaeolum*, *Primula*, and *Impatiens* cotyledons gave a violet reaction with I_2KI and 26 percent H_2SO_4 . These three cotyledons are those reported by Reiss (24) to contain amyloid. A distinctive reaction for amyloid may prove to be that of I_2KI followed by 25–26 percent H_2SO_4 rather than I_2KI alone.

2. The effect of treatment with chloral hydrate upon the hemicelluloses as shown by the iodine tests (table 2) indicates that the results obtained after previous treatment with chloral hydrate were the same as those obtained after a previous treatment with water. It will be shown later, however, that previous treatment with chloral hydrate greatly affects the solubility of hemicelluloses.

TABLE 2. *Color Reactions of Hemicelluloses of Endosperm and Cotyledon Cell Walls**

| | Phloro- glucin or Orcinol Followed by Concent- rated HCl and Heated | Chlor- zinc Iodid | I_2KI | I_2KI Followed by H_2SO_4 . Least Concen- tration of H_2SO_4 to Give Positive Reaction also Stated | Chloral Hydrate 18 Hrs. Washed in H_2O Followed by I_2KI 50% H_2SO_4 |
|------------------------------|--|-------------------------|---------|--|---|
| <i>Iris</i> | | | | | |
| <i>pallida</i> | V† | — | — | — | — |
| <i>Asparagus</i> | | | | | |
| <i>Sprengeri</i> | G† | — | — | — | — |
| <i>Diospyros</i> | | | | B with | |
| <i>virginiana</i> | — | — | — | 50% H_2SO_4 | + |
| <i>Phoenix</i> | | | | B with | |
| <i>dactylifera</i> | V† | V | — | 50% H_2SO_4 | + |
| <i>Coffea</i> | | | | B with | |
| <i>arabica</i> | V† | V | — | 50% H_2SO_4 | + |
| <i>Strychnos</i> | | | | B with | |
| <i>nux-vomica</i> | V† | V | — | 50% H_2SO_4 | + |
| <i>Lupinus</i> | | | | B with | |
| <i>hirsutus</i> | V† | P | — | 50% H_2SO_4 | + |
| <i>Tropaeolum</i> | | | | B without | |
| <i>majus</i> | V† | V | B | H_2SO_4 | + |
| <i>Primula</i> | | | | V with | |
| <i>officinalis</i> | V† | — | — | 26% H_2SO_4 | + |
| <i>Impatiens</i> | | | | V with | |
| <i>balsamina</i> | V† | — | — | 26% H_2SO_4 | + |

* The minus sign indicates a negative reaction; the plus sign, a positive reaction; V, a violet color; B, a blue color; P, pleochroism. In the last column but one the percent of H_2SO_4 required to give a positive reaction is stated. In all cases the seeds were subjected to a previous soaking in water from one to three days.

† V, positive for pentoses. G, positive for hexoses.

Solubility Experiments

In contrast to the color reaction tests which were made on slides, all solubility tests were made in Syracuse watch glasses. Greater uniformity of experimental conditions for both short and long time experiments could be obtained by the Syracuse watch glass method. There was no mechanical pressure such as that of the cover slip which was found markedly to

increase the speed of reaction when a slide was used. Also the concentration of the solutions remained more uniform and solution or solvent could be added from time to time.

The sections were examined either in the watch glass or on a slide. The latter method was used when the sections were not too crumbly and when the fumes of the reagents might prove injurious to the microscope. In a number of the experiments the sections were removed from time to time, washed, and treated for hydrocellulose. This reaction was used to make the wall layers distinguishable when the walls were comparatively thin. When the walls were thin and transparent a difficulty in focusing occurred and there was a possibility of mistaking the swollen primary or secondary walls for the whole wall. However, with most reagents, the entire section disappeared or broke up into fragments indicating that the primary wall dissolved as rapidly as or more rapidly than the rest of the wall. This fragmentary condition was not used as a standard of solubility unless no wall layers could be distinguished in the fragments. If the tertiary wall was not completely disintegrated in 30 days it was said to be insoluble.

1. Solubility of Cell Walls of Endosperms and Cotyledons in Cellulose and Hemicellulose Solvents

Copper Oxid in Ammonia. Poulson (23) cites Schweitzer, as reporting that pure cellulose swells and is dissolved in $\text{Cu}(\text{OH})_2$ in NH_4OH without conversion into amyloid. Kabsch (17) claims that $\text{Cu}(\text{OH})_2$ in NH_4OH is a reagent for pectose. When a tissue containing pectose is treated with the reagent a fine skeleton of cupric pectate is left behind. Van Wisselingh (37) says that many hemicelluloses dissolve in $\text{Cu}(\text{OH})_2$ in NH_4OH . Eckerson (10) reports that only cellulose and hemicellulose dissolve in this reagent. We may conclude that if the walls disappear in $\text{Cu}(\text{OH})_2$ in NH_4OH the cellulose or hemicellulose was the chief constituent of the walls.

Table 3 shows that all the tertiary walls are soluble in $\text{Cu}(\text{OH})_2$ in NH_4OH but that the speed of solution varies. A previous treatment with chloral hydrate greatly increased the rate of solution in every case. These solubility reactions indicate that the tertiary wall is composed of hemicellulose, cellulose, or a combination of both.

Boiling Glycerine. Schorger (28) quotes Honig as saying that glycerine at 210°C . will dissolve hemicelluloses. Shaw (34) determined that cellulose occurring in the palisade cells of fruit coats of *Nelumbo lutea* did not dissolve in glycerine boiled at 281°C . for 30 minutes. Schneider and Zimmerman (27) consider hemicelluloses soluble in glycerine at 210°C . while Van Wisselingh says that the amyloids of seeds of *Peonia officinalis* and *Tamarindus indica* are not wholly dissolved at 300°C . The effect of glycerine therefore requires some study. The tests were carried on in small beakers.

Table 3 shows that all tertiary walls except those of the endosperm of

Diospyros and of *Strychnos* are soluble in glycerine boiled for 30 minutes or less. This test may therefore be an aid in distinguishing hemicellulose from cellulose. The tissue remaining after treatment with hot glycerine is very transparent.

TABLE 3. *Solubility Reactions of Hemicelluloses in Cu(OH)₂ in NH₄OH and in Boiling Glycerine*

| | Time Required for Solution of Tertiary Walls in CuO in NH ₄ OH after Previous Treatments in: | | Time Required for Solution in Glycerine after Previous Treatment in H ₂ O |
|------------------------------|--|------------------------------|--|
| | H ₂ O 1 to 3 Days | Chloral Hy- drate 18 Hrs. | 1 to 3 Days |
| <i>Iris</i> | + | + | + |
| <i>pallida</i> | 10 hrs. | 25 mins. | Boil 15 mins. |
| <i>Asparagus</i> | + | + | + |
| <i>Sprengeri</i> | 9 hrs. | 2 1/4 hrs. | Boil 15 mins. |
| <i>Diospyros</i> | + | + | — |
| <i>virginiana</i> | 3 wks. | 1 3/4 hrs. | Boil 1 hour |
| <i>Phoenix</i> | + | + | + |
| <i>dactylifera</i> | 4 days | 10 mins | Boil 30 mins. |
| <i>Coffea</i> | + | + | + |
| <i>arabica</i> | 2 days | 6 mins. | Boil 30 mins. |
| <i>Strychnos</i> | + | + | — |
| <i>nux-vomica</i> | 4 days | 3 mins. | Boil 1 hour |
| <i>Lupinus</i> | + | + | + |
| <i>hirsutus</i> | 2 days | 30 mins. | Boil 30 mins. |
| <i>Tropaeolum</i> | + | + | + |
| <i>majus</i> | 1 day | 40 mins. | Boil 15 mins. |
| <i>Primula</i> | + | + | + |
| <i>officinalis</i> | 2 days | 40 mins. | 250 C. |
| <i>Impatiens</i> | + | + | + |
| <i>balsamina</i> | 5 days | 30 mins. | 210 C. |

Zinc Chlorid and Hydrochloric Acid (1 : 2 by weight). Cross and Bevan (8) report this reagent as a solvent for cellulose and hemicellulose. Its reactions on other substances are not known. Table 4 shows that all the hemicelluloses except those of *Diospyros* and *Phoenix* endosperms are soluble in ZnCl₂ and HCl. In general a previous treatment with chloral hydrate increases the rate of reaction.

Chromic Acid. Poulsen (23) claims that this reagent dissolves all substances except silicified and corky layers. It was employed in this study to determine whether suberin or cutin are present in the cell walls. Table 4 shows that all the tertiary walls were soluble in chromic acid. However, an oily substance was found on the slide after the remainder of the cell walls of endosperms of *Diospyros* disappeared. This indicates the presence of a lipid, suberin or cutin, which may be the cause of the comparative resistance of the tertiary wall of *Diospyros* endosperm to other cellulose and hemicellulose solvents.

TABLE 4. *Solubility Reactions of Hemicelluloses in ZnCl₂ and Concentrated HCl and in Chromic Acid**

| | Time Required for Solution in ZnCl ₂ and Concentrated HCl (1 : 2 by wt.) after Previous Treatments in: | | Time Required for Solution in 50% Chromic Acid after Previous Treatments with H ₂ O 1 to 3 Days |
|------------------------------|--|------------------------------|--|
| | H ₂ O 1 to 3 Days | Chloral Hy- drate 18 Hrs. | |
| <i>Iris</i> | + | + | + |
| <i>pallada</i> | 5 mins. | 5 mins. | 25 mins. |
| <i>Asparagus</i> | + | + | + |
| <i>Sprengeri</i> | 3 hrs. | 15 mins. | 25 mins. |
| <i>Diospyros</i> | — | — | + |
| <i>virginiana</i> | 42 days. | 20 days | 25 mins. |
| <i>Phoenix</i> | — | + | + |
| <i>dactylifera</i> | 68 days | 12 days | 30 mins. |
| <i>Coffea</i> | + | + | + |
| <i>arabica</i> | 2 days | + | 35 mins. |
| <i>Strychnos</i> | + | + | + |
| <i>nux-vomica</i> | 45 mins. | 30 mins. | 10 mins. |
| <i>Lupinus</i> | + | + | + |
| <i>hirsulus</i> | 10 mins. | 3 mins. | 12 mins. |
| <i>Tropaeolum</i> | + | + | + |
| <i>majus</i> | 3½ hrs. | 1 min. | 15 mins. |
| <i>Primula</i> | + | + | + |
| <i>officinalis</i> | 5 mins. | + | 10 mins. |
| <i>Impatiens</i> | + | + | + |
| <i>balsamina</i> | 3½ hrs. | 10 mins. | 1 min. |

* Plus sign indicates a positive reaction and minus sign a negative reaction.

2. Solubility of Tertiary Walls of Endosperms and Cotyledons in Javelle Water and in Hydrogen Peroxid

Javelle Water. This reagent is used because of the free chlorine in the solution. During the oxidation process according to Cross and Bevan (8) lignin is removed from the walls in which it occurs leaving any cellulose with which it might be associated still in the walls. Table 5 shows that all the tertiary walls studied except those of *Coffea* and *Strychnos* are soluble in Javelle water in from 1 to 26 days. Hemicelluloses may not, therefore, be distinguished from lignin by the Javelle water reaction. A previous treatment with chloral hydrate increases the rate of reaction of Javelle water upon all the tertiary walls except that of *Asparagus* and *Phoenix* endosperms.

Hydrogen Peroxid. This reagent is also used for an oxidation agent for lignin according to Eckerson (10). In testing for pectic substances in the primary walls of *Tropaeolum* cotyledon with 3 to 6 percent H₂O₂ (heated on the sections in a sealed watch glass at 60° C. for two hours), it was found that the hemicellulose disappeared after standing several days in the cold. The effect of H₂O₂ upon the other hemicelluloses was, therefore, determined. Table 5 shows that some hemicelluloses are soluble in H₂O₂ in

2 to 10 days. A previous treatment with chloral hydrate does not consistently increase the rate of reaction of H_2O_2 upon the tertiary walls.

TABLE 5. *The Solubility Reactions of Hemicelluloses in Javelle Water and Hydrogen Peroxid*

| | Javelle Water after Previous Treatments in: | | Hydrogen Peroxid at 60° C. for 2 Hours after Previous Treatments in: | |
|--------------------------|---|--------------------------------|---|--------------------------------|
| | H ₂ O 1 to 3 Days | Chloral Hydrate 18 Hours | H ₂ O 1 to 3 Days | Chloral Hydrate 18 Hours |
| <i>Iris</i> | + | + | + | + |
| <i>pallida</i> | 5 days | 20 hours | 9 days | 9 days |
| <i>Asparagus</i> | + | + | + | — |
| <i>Sprengeri</i> | 8 days | 9 days | 9 days | 46 days |
| <i>Diospyros</i> | + | + | — | — |
| <i>virginiana</i> | 26 days | 20 days | | 81 days |
| <i>Phoenix</i> | + | + | | + |
| <i>dactylifera</i> | 6 days | 11 days | | 6 days |
| <i>Coffea</i> | — | + | — | — |
| <i>arabica</i> | 31 days | | | |
| <i>Strychnos</i> | — | + | | + |
| <i>nux-vomica</i> | 51 days | 9 days | | |
| <i>Lupinus</i> | + | + | + | |
| <i>hirsutus</i> | 5 days | 24 hours | 2 days | |
| <i>Tropaeolum</i> | + | + | + | + |
| <i>majus</i> | 5 days | 20 hours | | 14 days |
| <i>Primula</i> | + | | + | |
| <i>officinalis</i> | 15 days | | 7 days | |
| <i>Impatiens</i> | + | + | + | + |
| <i>balsamina</i> | 2 days | 2½ hours | 10 days | 36 hours |

3. The Solubility of Tertiary Walls of Endosperms and Cotyledons in Schulze's Reagent and Pectic Solvents

Schulze's Reagent. (Concentrated potassium chlorate solution and concentrated nitric acid 1 : 1.) Poulsen (23) says this reagent may be used for the destruction of the middle lamella, especially of wood. Schulze and Tollens (33) claimed that hemicelluloses are insoluble in Schulze's reagent. Table 6 shows that all the hemicelluloses studied in this research with the exception of that in the cotyledons of *Lupinus* are soluble in Schulze's reagent.

Hydrochloric Acid (2 percent). Heat gently for half to one hour. Follow with 2 percent *Potassium Hydroxid* in the cold. This method is recommended by Eckerson (10) for removing all pectic substances. The reputed similarity of the hemicelluloses to pectic substances made it seem advisable to try this test upon the hemicelluloses. Table 6 shows that the hemicelluloses of *Primula* and *Lupinus* cotyledons are dissolved when heated in 2 percent HCl at 80° C. for 1½ hours and then treated with cold 2 percent KOH.

Crystallographic Properties

Sections of all the tissues under investigation were dried at 100° C. for one hour and then studied in polarized light between crossed nicols. The tertiary walls of the treated tissues were observed to be doubly re-

TABLE 6. *Solubility Reactions of Hemicelluloses of Cell Walls of Endosperms and Cotyledons in Schulze's Reagent and in Pectic Solvents*

| | Time Required for Solution in Schulze's Reagent after Treatment in: | | Treated with 2-5% HCl at 80° C. for 1½ Hours and Transferred to Cold 2% KOH after Treatment in H ₂ O 1 to 3 Days |
|-----------------------------------|---|--------------------------|---|
| | H ₂ O 1 to 3 Days | Chloral Hydrate 18 Hours | |
| <i>Iris pallida</i> | + | + | — |
| | 5 days | 42 hours Jelly-like | |
| <i>Asparagus Sprengeri</i> | + | + | — |
| | 3 days | 20 hours | |
| <i>Diospyros virginiana</i> | + | + | — |
| | 13 days* | 20 days* | |
| <i>Phoenix dactylifera</i> | + | + | — |
| | 18 days* | 10 days* | |
| <i>Coffea arabica</i> | + | + | — |
| | 18 days* | 9 days | |
| <i>Strychnos nux-vomica</i> | — | — | |
| | 15 mins. | 54 hours Jelly-like | |
| <i>Lupinus hirsutus</i> | + | + | + |
| | 26 days | 1 hour | 2 days |
| <i>Tropaeolum majus</i> | + | + | |
| | 4½ days* | 20 hours | |
| <i>Primula officinalis</i> | + | + | + |
| | 8 days | 3 hours | 1 day |
| <i>Impatiens balsamina</i> | + | + | — |
| | 10 mins. | 30 mins. | |

* These tests are reported as having a positive reaction since the walls no longer give the hydrocellulose reaction.

fractive (see table 7). The property of double refraction is characteristic of crystallized material, and can be used, when it occurs, as a criterion for the presence of crystalline bodies except in those cases where the double refraction may be due to a directional strain, as in the drying of a colloid. The occurrence of double refraction in the cell walls of plants may be the result of the presence either of aggregates of crystalline substances or of colloids under strain.

Material which does not have the outward form of a crystal may be composed of an aggregate of tiny crystallized particles interwoven in such a way as to conceal the crystalline form. When these aggregates are dispersed and each discrete particle studied separately, the optical properties can be measured and these used for purposes of identification.

In very tiny crystals the outward form of the crystal may be absent, due to the fact that the surface tension of the crystal is so large compared to the rigidity of such a tiny particle, and the particle may assume the form of a spherule or a cylindrical particle called a trichite.

Refractive Properties

In the drying of amorphous material, such as colloids, doubly refractive properties are often observed, and by the use of a selenite plate in polarized light it is possible to evaluate the amount of stress and the character of the

TABLE 7. *Anisotropy of the Hemicelluloses of Endosperm and Cotyledon Cell Walls**

| | Sections Untreated | After Previous Treatments in: | | |
|--------------------------|-----------------------|------------------------------------|---|--------------------------------|
| | | H ₂ O 1 to 3 Days | H ₂ O, 1 to 3 Days. Then 100° C. 1 Hr. | Chloral Hydrate 18 Hours |
| | Mounted in Oil | Mounted in H ₂ O | Mounted in Oil | Mounted in H ₂ O |
| <i>Iris</i> | | | | |
| <i>pallida</i> | + | — | + | — |
| <i>Asparagus</i> | | | | |
| <i>Sprengeri</i> | + | — | + | W |
| <i>Diospyros</i> | | | | |
| <i>virginiana</i> | + | — | + | W |
| <i>Phoenix</i> | | | | |
| <i>dactylifera</i> | + | + | + | + |
| <i>Coffea</i> | | | | |
| <i>arabica</i> | + | + | + | + |
| <i>Strychnos</i> | | | | |
| <i>nux-vomica</i> | + | + | + | — |
| <i>Lupinus</i> | | | | |
| <i>hirsutus</i> | W | — | + | — |
| <i>Tropaeolum</i> | | | | |
| <i>majus</i> | + | — | + | W |
| <i>Primula</i> | | | | |
| <i>officinalis</i> | + | — | + | — |
| <i>Impatiens</i> | | | | |
| <i>balsamina</i> | + | — | + | — |

* + indicates anisotropy; — indicates isotropy; W indicates weakly anisotropic

stress in a certain direction; that is, to determine if the body is under a strain due to tension or to compression.

For the cell walls under discussion it is difficult to interpret the birefringence and the variable character of optical elongation on the basis of strain phenomena, and in the absence of a complete array of all the facts it seems best to discuss the phenomenon as one involving the presence of a crystalline substance. Such an explanation seems at this time to fit the facts better than one based on strain, but a complete discussion of wall structure should consider both phenomena.

The tertiary walls of endosperms of *Asparagus*, *Iris*, and *Phoenix* and of cotyledons of *Tropaeolum* contain pits almost cylindrical in shape (inter-cellular canals) which extend to the secondary walls in depth. When the inner surface of the cell wall is observed between crossed nicols the pits are seen as round apertures. Little or no interference color is observed within the pits either because of the absence or of the thinness of the septum of crystalline material covering the middle lamella. Immediately surrounding the pits there is an area of high interference color which gradually fades out in the spaces between the pits. Dark crosses are visible whose centers are the pits. The dark bars of the cross are always parallel to the vibration directions of the nicols of the microscope. This dark cross is characteristic of a radial crystal arrangement; the interference color and the extinction in the form of a cross indicate that these crystals radiate from the sides of the pits and that as the crystals are projected from the sides of the pits they become separated from each other by a greater distance.

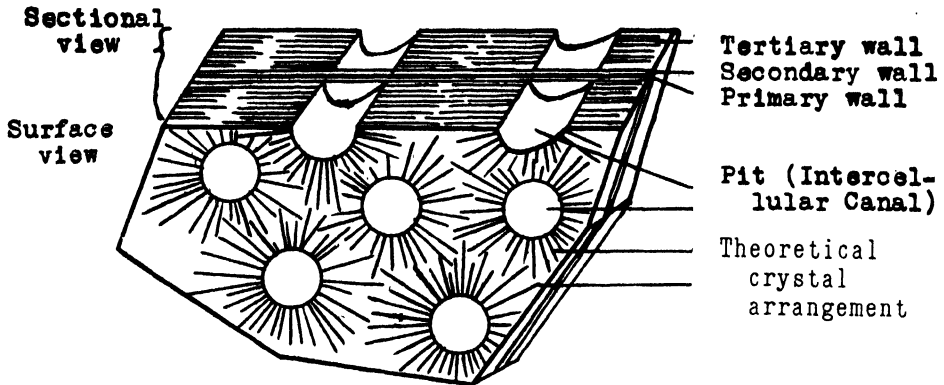
By means of the selenite plate the crystals radiating from the pits appear to have negative elongation in the surface view orientation. If a selenite plate is inserted in the microscope, those crystals of the aggregate which have their axis of elongation parallel to the vibration directions of the ray with the high index in the selenite plate show a yellow color; those crystals which have their axis of elongation at right angles to the high index ray in the plate show blue color.

In sectional views of the wall the crystals would be in a position at right angles or almost at right angles to that position in which they occur in the surface view just described. In this orientation the crystals have positive elongation demonstrated as follows. An uncurved wall shows parallel homogeneous extinction, which means that the vibration direction of one of the two rays transmitted through the wall in this orientation is parallel to the plane of the wall and that the vibration direction of the second ray is perpendicular to that of the first ray. By means of the selenite plate it may be shown that the ray which vibrates in the direction parallel to the plane of the cell wall has a higher index of refraction than the ray which vibrates in the direction perpendicular to the cell wall. This fact indicates that the crystals composing the cell walls have a positive elongation when examined in the sectional view. Since the extinction of the sectional view is homogeneous the crystals are parallel to each other and since the extinction of the sectional view occurs only when the edges of the sectioned wall are parallel to the nicols no matter at what angle the surface view section is cut, the crystals are oriented parallel to the plane of the cell wall.

Crystals that have negative elongation when in one position and a positive elongation when viewed at right angles to that position are bi-axial crystals. Since the extinction of the sectional view occurs when the edges of the sectioned wall are parallel to the nicols no matter at what

angle to the surface view the section is cut it is probable that the crystals are orthorhombic. The ray which vibrates in a direction parallel to the elongation of the crystal is the one with the medium index of refraction, β . Since β is the ray with the higher index of refraction in the sectional view orientation, the ray vibrating perpendicular to β in that orientation must be the ray with the lowest index, α . The ray whose vibration direction is perpendicular to β or the direction of elongation in the surface view orientation must be the ray with the highest index, γ .

In conclusion it appears that the tertiary walls may be composed of crystal aggregates, and that the biaxial crystals forming these aggregates



TEXT FIG. 1. Diagram of theoretical crystalline structure.

radiate from the pits. Text figure 1 illustrates the theory of the radial crystal arrangement.

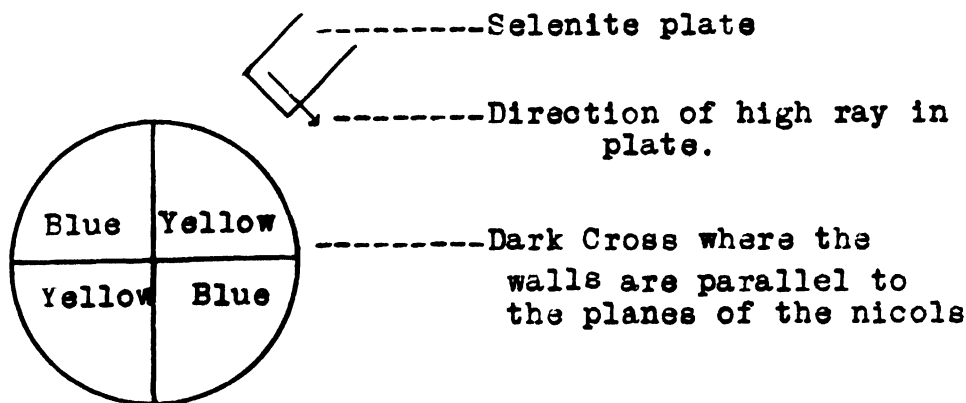
The pits if present in the cell walls of endosperms of *Diospyros*, *Coffea*, and *Strychnos* and of cotyledons of *Impatiens* have either very small diameters or are very irregular in shape. Intercellular connections in endosperms of *Diospyros* have been demonstrated by Chamberlain (6) to be fine threads of protoplasm through canals of very small diameter. Intercellular canals of small diameter have been reported for *Strychnos* endosperms by Gilson (13). The apertures of pits are not visible in surface views of cell walls of these species and it is impossible to demonstrate a crystalline arrangement by the selenite plate method described above. Nor can it be stated at present whether the crystals of the tertiary walls of these species are uniaxial or biaxial. However, in sectional views the cell walls of these species have the same optical properties as those described for *Iris*, *Asparagus*, *Phoenix*, and *Tropaeolum*.

The cell walls of cotyledons of *Lupinus* appeared faintly yellow in cross sections when the selenite plate was inserted, regardless of the orientation of the walls in relation to the high ray in the plate. This faint homogeneous yellow color is consistent with the fact that the walls are weakly anisotropic even when dried.

A radiating arrangement of the walls of the cells of endosperms of *Iris*.

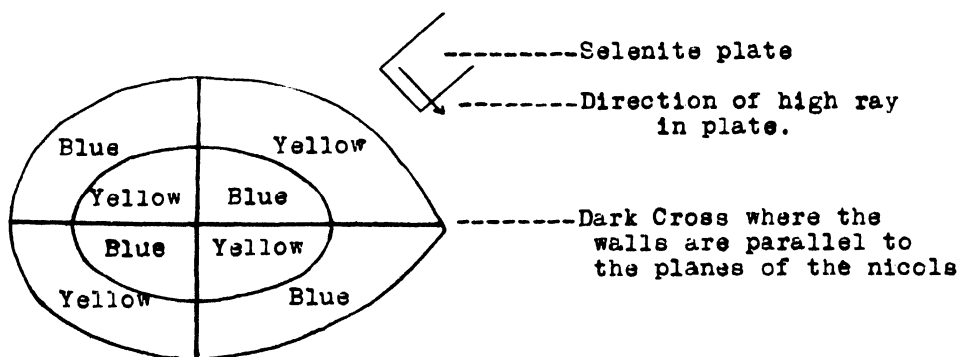
Asparagus, *Phoenix*, and of cotyledons of *Primula* and *Impatiens* may be demonstrated with the selenite plate. This radiation starts at the center of the seed and extends to the outer cells of the endosperms or cotyledons. The selenite plate effect is illustrated by text figure 2.

The sections of endosperm of *Diospyros* show a somewhat different



TEXT FIG. 2. Diagram of cross section through center of endosperm in which cells have radiating arrangement, as shown by blue and yellow quadrants with selenite plate.

effect. The walls of the cylindrical cells of the endosperm next the seed coat have the same orientation of crystalline material as demonstrated by text figure 2. But near the center of the section the cell shape is irregular rather than cylindrical and the tertiary wall appears as irregular thickenings of anisotropic substance against the secondary wall. The elongation of the crystals forming these thickenings would appear to be perpendicular



TEXT FIG. 3. Diagram of cross section through center of endosperm of *Diospyros*. Blue and yellow portions as they appear under selenite plate.

to the elongation of those forming the cylindrical cell walls. The selenite plate appearance is illustrated by text figure 3.

The radiating arrangement of the cells is not apparent in sections of

endosperms of *Strychnos* and *Coffea* and of cotyledons of *Tropaeolum* even when the sections are cut close to the center of the seed.

In connection with the crystalline structure discussed above, the development of the endosperm of *Asparagus* and *Iris* as studied microchemically is interesting. Berries of *Asparagus* and seed pods of *Iris* were collected in different stages of development, which, for convenience in discussion, may be divided into four distinct stages. In the youngest the endosperms were composed mostly of liquid, scarcely more viscous than water, and the cell walls were very thin. In the second stage the endosperm had a jelly-like consistency. In the third stage it was firm but not horny as in the fourth and mature stage. The tertiary wall was formed between the second and third stages described. Osazone tests made on the first two stages were positive, a very copious precipitate being formed. Since the osazone tests were carried on at a low temperature (45° C.) and the crystals formed in 12 to 18 hours and possessed the characteristic aggregate structure of glucosazones, they were probably from glucose or fructose. No hydrazones were observed in these tests. Osazone tests made on stages three and four were negative. Attempts to find stages intermediate to the second and third were unsuccessful. In the third stage the walls are just as thick as they are in the fourth, and the intercellular canals are already formed.

Indices of Refraction

The sections were cut and washed as for previous tests. Then they were dried in Syracuse watch glasses at 100° C. for one hour. An attempt was made to determine the indices as soon as possible after removing the sections from the oven. There was some indication that the indices changed somewhat after the sections had stood in oil for some time. Therefore the sections were mounted only as needed, the Syracuse watch glass being

TABLE 8. *Indices of Refraction of the Hemicelluloses of Endosperm and Cotyledon Cell Walls**

| | Sections Kept in Water 1 to 3 Days, then Dried at 100° C. for 1 Hour. | |
|-----------------------------------|--|---------------------------------------|
| | Value for α | Value Between β and γ |
| <i>Iris pallida</i> | 1.528 | 1.538 |
| <i>Asparagus Sprengeri</i> | 1.524 | 1.538 |
| <i>Diospyros virginiana</i> | 1.528 | 1.535 |
| <i>Phoenix dactylifera</i> | 1.528 | 1.545 |
| <i>Strychnos nux-vomica</i> | 1.528 | 1.533 |
| <i>Tropaeolum majus</i> | 1.523 | 1.538 |

* On those walls for which two indices were determined it was possible to get an accurate value for α , but the other index determined in each case is probably an intermediate value between β and γ because of the orientation of the crystals.

kept covered to prevent hygroscopic action meanwhile. Mixtures of paraffin oil (index -1.4610) and Halowax (index -1.6317) were used as refractive media.

Indices could be determined by the Becke line method on the sectional views of all the tissues. A summary of the indices determined is given in table 8.³

Shaw (34) found that the indices of refraction for cellulose of walls of palisade cells of fruit coats of *Nelumbo lutea* were $\alpha = 1.531$, $\beta = 1.538$, $\gamma = 1.568$. The value of α for the hemicelluloses studied varies from .003 to .008 lower than that found for cellulose according to Shaw. Anderson (2) gave two indices for the cellulose of the bast fibers of flax, *i.e.*, 1.52 and 1.58. The indices were taken, however, after the material was hydrolized slightly, washed, and dried on a hot plate.

Only one index was obtained in the following cases:

| | | | |
|-------------------------|---------|----------------------------|---------|
| <i>Coffea arabica</i> | = 1.528 | <i>Primula officinalis</i> | = 1.521 |
| <i>Lupinus hirsutus</i> | = 1.530 | <i>Impatiens balsamina</i> | = 1.521 |

CONCLUSIONS

I. From the color reactions

1. Of the ten species studied, the hemicellulose of cotyledons of *Tropaeolum majus* alone colors blue with I_2KI , when in the natural state.

2. Hemicelluloses of cotyledons of *Tropaeolum majus*, *Primula officinalis*, and *Impatiens balsamina* are colored violet with I_2KI and 26 percent H_2SO_4 . These hemicelluloses occur in seeds which have been reported to contain amyloid.

3. Hemicelluloses of endosperms of *Diospyros virginiana*, *Phoenix dactylifera*, *Strychnos nux-vomica*, *Coffea arabica*, and cotyledons of *Lupinus hirsutus* are colored blue with I_2KI and 50 percent H_2SO_4 , but not when a lower concentration of acid is used.

4. Some celluloses are also colored blue by I_2KI and 50 percent H_2SO_4 . Therefore these reagents may not be used to distinguish hemicellulose from cellulose.

5. Hemicelluloses of endosperms of *Iris pallida* and *Asparagus Sprengeri* are not colored blue by I_2KI and H_2SO_4 of any concentration when in the natural state.

6. Hemicelluloses of endosperms of *Iris pallida*, *Asparagus Sprengeri*, and *Diospyros virginiana*, and of cotyledons of *Primula officinalis* and *Impatiens balsamina*, are not colored by the chlorzinc-iodid reagent.

7. The hemicelluloses of endosperms of *Coffea arabica*, *Phoenix dactylifera*, and *Strychnos nux-vomica* and of the cotyledons of *Lupinus hirsutus* and *Tropaeolum majus* are colored blue by the chlorzinc-iodid reagent. Of these only the colored walls of the cotyledons of *Lupinus* seemed to be pleochroic.

8. All the hemicelluloses studied except those of endosperms of *Aspara-*

³ Brown (4) determined the refractive indices of cell walls of *Phoenix dactylifera* endosperm as being from 1.526 to 1.540.

gus Sprengeri and *Diospyros virginiana* have a violet color when heated with phloroglucin or orcinol and concentrated HCl. The presence of pentosans is indicated.

9. The hemicellulose of *Asparagus Sprengeri* and *Coffea arabica* have a gold color when heated with phloroglucin or orcinol and concentrated HCl. The presence of hexosans is indicated.

II. From the solubility reactions

1. The hemicelluloses of the ten tissues studied dissolved in all the solvents which have been reported to dissolve both cellulose and hemicellulose.

2. The complete solubility of the tertiary wall in $\text{Cu}(\text{OH})_2$ in NH_4OH indicates that hemicellulose or cellulose or a combination of both is present.

3. The hemicellulose of the tertiary walls of the endosperms of *Asparagus Sprengeri*, *Diospyros virginiana*, and *Phoenix dactylifera*, and of the cotyledons of *Tropaeolum majus* dissolved very much more quickly in the hemicellulose and cellulose solvents than the secondary walls.

4. Hemicelluloses of all the tertiary walls except those of endosperms of *Diospyros virginiana* and *Strychnos nux-vomica* dissolved when boiled in glycerine half an hour.

5. Glycerine may prove to be a reagent to use in distinguishing between cellulose and hemicelluloses since pure cellulose does not dissolve in boiling glycerine.

6. The hemicelluloses of all the tissues studied except those of endosperms of *Coffea* and *Strychnos* were dissolved in from 1 to 26 days by Javelle water. Javelle water may not therefore be used to distinguish between hemicelluloses and lignin.

7. Hemicelluloses of tertiary walls of endosperms of *Iris pallida* and *Asparagus Sprengeri*, and of cotyledons of *Lupinus hirsutus*, *Tropaeolum majus*, *Primula officinalis*, and *Impatiens balsamina* dissolved in H_2O_2 in from 2 to 10 days. H_2O_2 may not, therefore, be used to distinguish between hemicelluloses and pectic compounds.

8. Hemicelluloses of all the seeds were soluble in Schultze's reagent. As cellulose is reported not to be soluble in this reagent it may prove to be a means of distinguishing hemicellulose from cellulose.

9. The hemicelluloses of the cell walls of the cotyledons of *Lupinus hirsutus* and *Primula officinalis* were dissolved by the following treatment: 2-5 percent HCl heated at 80°C . for one and one-half hours followed by cold 2 percent KOH. This treatment will not, therefore, distinguish between hemicellulose and pectic materials.

III. From the study of the optic properties

1. The fact that all the tertiary walls studied are doubly refractive indicates that they are composed of an aggregate of anisotropic crystals.

2. From a study of optical properties a theory was developed that the tertiary walls of cells of endosperms of *Iris pallida*, *Asparagus Sprengeri*, and *Phoenix dactylifera* are composed of radial crystal aggregates.

3. The tertiary walls of all the tissues studied except that of *Lupinus hirsutus* have a positive elongation, indicating that crystals are so oriented in the wall that the vibration direction of the higher ray transmitted through the wall is parallel to the elongation of the wall.

4. The index of refraction of the lowest ray ($\alpha = 1.521$) for each of the tertiary walls studied is lower than the index of refraction for α of cellulose as determined by Shaw (34).

5. Intermediate indices of refraction for the rays β and γ were determined for the crystals of the tertiary walls of endosperms of *Iris pallida*, *Asparagus Sprengeri*, *Strychnos nux-vomica*, *Diospyros virginiana*, and *Phoenix dactylifera* and of cotyledons of *Tropaeolum majus*.

The writer wishes to acknowledge her indebtedness to Dr. H. C. Sampson and Dr. J. D. Sayre of the Department of Botany of the Ohio State University for many helpful suggestions and criticisms of the microchemical study; and to Dr. R. C. Burrell of the Department of Agricultural Chemistry of the same university for suggestions and assistance in the study of the hydrolytic products of the hemicelluloses; and to express her appreciation of the help and criticism of Dr. W. J. McCaughey of the Department of Mineralogy of the same university in the study of the optical properties.

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A PRELIMINARY REPORT ON THE CHROMOSOME COMPLEMENT OF "RABBIT-EARED ROGUES" IN CULINARY PEAS (*PISUM SATIVUM* L.)¹

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INTRODUCTION

Bateson and Pellew (1915, 1920) first reported the inheritance of the narrow-stipuled mutation or "rabbit-eared rogue"² in culinary peas. They concluded that the mutation acted as a dominant, though there was a failure of the expected segregation of rogue and non-rogue types. Certain intermediate forms were found also, of which the offspring or progeny derived from the upper nodes showed increasing percentages of rogues.

Brotherton (1923, 1924) reported on the F_2 of a cross, Gradus rogue \times type plants of Mummy (a non-rogue-producing pea). In the F_2 and F_3 the ratio between the rogues and non-rogues could not be explained on the basis of any Mendelian ratio. The number of rogues was much larger than the number of non-rogues. A postulation was made that this was due to the mutation of a factor for type, x , to the factor for rogue, X . When the composition of an individual was Xx the x was very unstable, and during the growth of an Xx individual mutation of x to X occurred so frequently that an Xx F_1 easily became largely XX at maturity.

In the Mummy strain the factor for non-rogue, x^1 , was an allelomorph of X , and was almost always more stable than x . x^1 rarely mutated to X when homozygous, but might at times mutate to X in Xx^1 individuals. Thus the numbers of x or x^1 and X gametes produced by an Xx or an Xx^1 hybrid would be unequal, and not according to any Mendelian ratio. This

¹ Papers from the Department of Genetics, Agricultural Experiment Station, University of Wisconsin, number 101. Published with the approval of the Director of the Station.

² A description of the "rabbit-eared rogue," on which this work was done, is given by Brotherton (1923). "Certain varieties of the garden pea, such as Early Giant, Ne Plus Ultra, and Duke of Albany, are characterized by broad, wavy stipules and leaflets, both having rounded, emarginate apices and broad, straight pods. Occasionally there occur in pure lines of such varieties plants called "rogues" that are described as "wild" or "vetch-like." The rogues differ from the parent form mainly in the reduction in width of the foliar parts (stipules and leaflets) and of the pods. The stipules of the rogues are narrow and pointed; the pods are narrow and curved along the upper suture. Rogues are not produced with any definite regularity or in such numbers that a characteristic ratio can be established between the rogues and parent form." To this may be added the facts that the rogue seeds used in this experiment were flattened in one plane, so becoming discoid, not spherical, and that when ripe they were somewhat green or mottled green and yellow in color, with an acrid taste.

type of mutation which occurs in more than one cell at the same time is called "mass somatic mutation" by Brotherton (1923).

Brotherton's results indicate that rogue crossed with type gives a rogue F_1 ; that non-rogue (as Mummy) crossed with type gives a non-rogue F_1 ; but that the ratio of rogue gametes to non-rogue gametes produced on a given plant (of heterozygous composition) was 1 : 1. This latter conclusion was drawn from the data "from the F_1 hybrid between a commercial variety known as Rice's 330 and rogue of self-fertilized progeny and progeny derived from backcrosses with Gradus type." Brotherton considers that any variation in percentage of rogues in the progeny of the upper nodes is due to further mass somatic mutation.

Pellew (1927) further confirms Brotherton's conclusion that rogue is dominant over type.

PROCEDURE

In the spring of 1927, through the kindness of W. Brotherton, Jr., and R. A. Brink, the author was provided with material from Brotherton's Gradus type and rogue stocks. It was hoped that further study and count of the chromosomes in rogue and type individuals might give some information concerning their constitution. In *Pisum sativum* the diploid number of 14 was determined by Němec (1903a, b, 1904), Strasburger (1907, 1911), Kemp (1910), Sakamura (1920), Wellensiek (1925), Dombrowskaja (1925), and Heitz (1926). A count of 7 for the haploid was reported by Cannon (1903), Strasburger (1911), and Winge (1925). The counts of Miss Thomas and K. Matsui (Bateson and Pellew, 1920) and of Winge (1925) indicated a haploid number of 7 for both Gradus type and rogue.

Seeds were germinated and when the hypocotyls had attained a length of 0.5 to 1.5 cm. they were removed and placed in fixative. Various fixing solutions were employed: Flemming's strong, medium, weak, chromacetic, Carnoy's, Benda's, and Bouin's. However, none of these gave a fixation suitable for the counting of chromosomes. It was not until the early part of 1928 that Licent's solution was tried (16 parts of 1 percent chromic acid : 3 parts of commercial formalin : 1 part of glacial acetic acid). This seemed to give a good cytological fixation, with the chromosomes especially clear.

Sections of 5 and 10 microns were made. Flemming's triple stain (safranin, gentian violet, and orange G) was used on some of the slides; Heidenhain's iron-alum haematoxylin was used on others. The latter seemed to be the better for chromosome counts.

Chromosome counts were made, as were several camera lucida drawings, with a magnification of 2400 times.

OBSERVATIONS

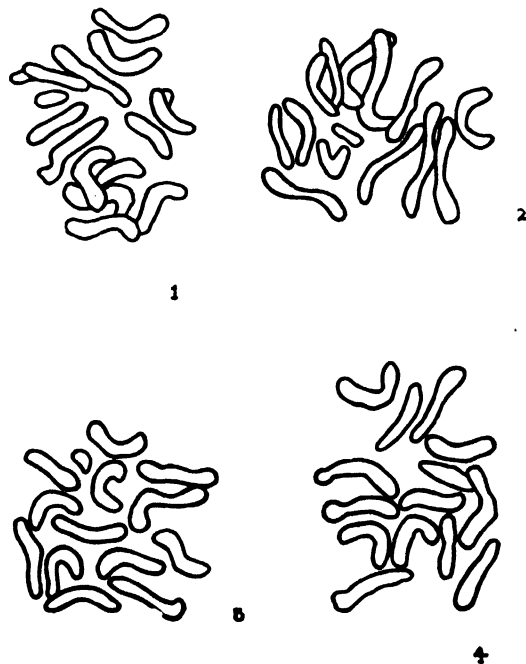
All counts were made from polar views of chromosomes in the metaphase. For the 14 counts of type material, lot 905-15 was used. The

number of chromosomes in each case was 14, as is shown in table 1. The counts for rogue were made on material taken from lots 900-12, 900-20, and 900-23. The records for these counts are also to be found in table 1.

TABLE 1. *Chromosome Counts in Root Tips of Rogue and Type Peas*

| | No. of Counts | 12 csm. | 13 csm. | 14 csm. | 15 csm. |
|--------------|---------------|---------|---------|---------|---------|
| 905-15 | 14 | | | 14 | |
| Total | 14 | | | 14 | |
| 900-12 | 13 | | 1 | 12 | |
| 900-20 | 1 | | | 1(?) | |
| 900-23 | 47 | 4 | 5 | 35-2(?) | 1 |
| Total | 61 | 4 | 6 | 47-3(?) | 1 |

Two camera lucida drawings were made of figures from rogue material and two of figures from type material (text figs. 1-4). In all cases the fourteen chromosomes may be clearly seen.



TEXT FIGS. 1-4. All drawings were made with the aid of a camera lucida. The magnification in each case was $\times 1500$. Figs. 1-2. Polar view of chromosomes in metaphase in cells of hypocotyl of *Gradus* type, lot 905-15. Figs. 3-4. Polar view of chromosomes in metaphase in cells of hypocotyl of *Gradus* rogue, lot 900-23.

DISCUSSION

The obvious conclusion is that the diploid chromosome number of *Gradus* peas, whether type or rogue, is 14, thus confirming the observations of Miss Thomas and Matsui, and of Winge. Apparent exceptions were probably due to the fact that not all the chromosomes lie in the same plane

and thus may be in such a position so as to be either entirely omitted from the section or so as to have their parts appear as separate chromosomes. In the former case counts of less than 14 would occur; in the latter, more than 14.

If Brotherton's hypothesis concerning mass mutation is to be applied here, it must be based, as Brotherton assumes, on some change other than chromosome number. Further work may show that a change has taken place in the rogue affecting the shape or size of individual chromosomes. However, the work, at present, is of too preliminary a nature for any statements to be made concerning this phase.

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LIESEGANG PHENOMENA IN FUNGI

ILLO HEIN

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The fascinating and familiar concentric distribution of fungous fruiting structures and the alternate concentric zones of dense and less dense mycelial aggregates have been many times reported in the literature but the data concerning the elusive factors which control them are too fragmentary and inadequate for a general theoretic treatment. It may be possible to relate the chemically produced Liesegang rings to the analogous appearances in fungi and other organic forms and to apply the explanations given for the Liesegang phenomena to the fungi.

Our present knowledge concerning periodic zonation in plants and inorganic matter is well summarized in the work of Küster (1913). Bisby (1925) recently briefly reviewed the literature on fungi and Kopacweski (1928) in a popular article summarizes the literature on the subject in general and calls attention to the many different forms of periodicity in nature. The circular distribution of fungi is perhaps best known in the fleshy forms and the reported observations have been adequately reviewed by Schantz and Piemeisel (1917).

In the lower fungi rings of sclerotia, conidia, ascocarps, or alternate zones of dense and less dense hyphal aggregates are well known. They appear most frequently in concentric, rhythmic succession and often closely resemble the Liesegang patterns. Milburn (1904), Molz (1907), Hutchinson (1907), Hedgcock (1906), Stevens and Hall (1909), Gallemaerts (1910), Riedemeister (1908), Munk (1912), and Bisby (1925) report studies on concentric zones produced in cultures of micro-fungi but aside from showing the important parts played by periodic temperature, light and moisture changes their studies throw little light on the elusive causal factors concerned with such fungi as are periodic under constant environmental conditions.

In my studies on fungi found associated with the commercial mushroom, I have grown species of *Sordaria*, *Chaetomium*, *Penicillium*, *Verticillium*, *Mycogone* and a yet unidentified sclerotium-producing fungus, common in mushroom beds, in a variety of different "Difco" agars, especially lima bean, nutrient, prune, and cornmeal as well as mushroom agar, horse-dung agar, and synthetic agar media all in various concentrations with some striking results from the point of view of the well known periodic phenomena.

The unidentified species of sclerotium produces a dark brown, usually

spherical sclerotium but in no case, even though several hundreds of cultures were made in numerous different media in various concentrations and under a variety of temperature and moisture conditions, was any kind of fruiting structure obtained from the sclerotia.

All the following cultures described are typical for the particular media used and unless otherwise mentioned, were grown under similar conditions of light and temperature, and the H-ion concentration was approximately 7.2. A number of examples of each culture studied were kept in continuous darkness and others under continuous illumination 2 feet away from a 100-watt electric light bulb with the daylight screened out. In no case was there any evidence of the effect of light or temperature on ring formation in these fungi.

The sclerotia, which are not in contact with each other, are quite spherical in form although here and there are individuals slightly ovoid in section with the long axis parallel to the substrate and still others which appear to have developed from the fusion in early stages of two or more single spheres. Throughout the structure the cells fit closely together without interstices and they have flat faces and are isodiametric and more or less alike in shape, except that in the outer single-celled peripheral layer they are somewhat flattened and pigmented. The majority of the cells are six-sided in section but this number may vary from four to eight. It would appear without further investigation that the cells may be typically the tetrakaidecahedra of Lord Kelvin. Lewis's contentions (1923, 1925, 1928), supported by Matzke (1927) and Gross (1927), that massed cells are typically of this form, may find further support in the pseudoparenchyma of this and other fungi.

In vertical section, the vegetative area of older cultures in which the medium is completely overgrown presents three distinct horizontal layers of mycelia. There are two layers of aerial mycelium of which the upper consists of loosely arranged, mostly radially growing hyphae. Interhyphal spaces in the upper layer are large and abundant but occasionally two or three hyphae adhere closely to one another showing a slightly synnematous tendency. The lower layer is distinctly separated from the upper and approximately as thick, but consists of a tightly interwoven plectenchyma of profusely branched irregularly growing hyphae. In this area the hyphal cells are shorter, more frequently bent in various directions, and variable in diameter from 5-10 microns with an average diameter of about 6 microns. In general appearance this plectenchyma is similar to that of *Sclerotinia sclerotiorum* (figured by de Bary, 1887, fig. 14). There is a gradual transition from this plectenchyma to the submerged mycelium. In the latter the cells become longer, growth becomes gradually less profuse, and most of the hyphae tend to grow in radial horizontal directions. From the very meager growth in the substrate and the profuse growth on

the surface of the substrate it would appear that the fungus requires an abundance of atmospheric oxygen.

In prune agar (20 grams in 1000 cc. water) a clear circular area (12 mm. in diameter) of radially growing vegetative hyphae entirely free from sclerotia is first produced and then very abruptly a 5-8 mm. wide ring of dark brown sclerotia is formed (Pl. VIII, fig. 2). The sclerotia in the ring are produced in great profusion over a very limited area and are often arranged as though piled up upon one another 5 and 6 high in the center of the mass.

The sharp demarcation between the ring of sclerotia and the vegetative area is striking but there is no evidence as to the causal factors concerned in sclerotial production either in the color or the form of the mycelium. The inner margin of the ring is somewhat more sharply delimited from the vegetative area than the outer margin but both margins are distinct. In this medium the vegetative area following the first ring of sclerotia is 6-8 mm. wide and in old cultures there are produced in it here and there a few sclerotia. Encircling the vegetative area a second ring is produced similar to the first in width and general appearance.

The aerial mycelium is the same in amount, branching, septation, and thickness of hyphae in the sclerotia-producing zones as in the purely vegetative areas and the submerged mycelium is similar in appearance throughout the culture.

In more concentrated prune agar media (25 grams to 1000 cc. water) a smaller area of vegetative mycelium one centimeter in diameter and circular in outline is produced about the center of inoculation (fig. 1). This is followed by an irregular ring of sclerotia about 5 mm. wide and then an 8 mm. wide ring of vegetative hyphae. A second sharply defined ring uniformly 5 mm. wide is laid down and is then followed by a circular vegetative area similar to the previous one. The third ring of sclerotia tends to be more irregular in outline on the outside border. There is a more gradual change from the sclerotial to the vegetative area. In the final vegetative area the sclerotia are somewhat irregularly scattered.

In lima bean agar a circular growth of vegetative hyphae 8 mm. wide is followed by an indefinite ring of sclerotia, which varies in width around 5 mm. Following this a very wide circular vegetative area 2.5 cm. wide is laid down and then an irregular one of sclerotia to the limits of the disc (fig. 4). Nowhere on the bean agar do the dense aggregates of sclerotia occur as they do in the prune agar cultures.

In cornmeal agar very irregular growth is produced. The dendritic branching of the vegetative hyphae to be described later is characteristic in this medium and sclerotia occur singly or in patches indefinitely distributed over the substrate (fig. 5).

Similar cultures were made in 20 x 200 mm. petri dishes to obtain larger colonies and to observe appearances in the larger rings (fig. 3). The

first formed rings are identical with those described in the smaller culture dishes but in the later formed rings the contrast between the sharpness of outline of the inner and outer margins is more conspicuous. The typical example shown in figure 4 is from an older culture and in it the central region has become somewhat overgrown with later profuse aerial growth which partly obscures the first three rings. Five rings in all were produced. The last three are still distinctly visible in the figure. The 4th and 5th rings have become progressively wider and tend gradually to a less profuse and more irregular growth on the outer margin. Thus the factors which initiate and control sclerotial production appear to begin abruptly and diminish gradually as the ring widens.

When grown in nutrient agar the fungus described produces abundant sclerotia but no rings (fig. 6). A characteristic dendritic branching with abundant sclerotia on every filament develops radially outward from the center of infection. There is a tendency in the hyphae to form main radii which consist of from a few to twenty or more parallel hyphae growing closely together but without adhesion. The grouping into bundles of hyphae forming main radii and secondary radii of individual filaments suggests an analogy with such cultures as are figured by Hartog (1927). The main radii do not form synnemata as in the basidiomycetes although they appear somewhat like the early stages of synnematus development in the common mushroom (Hein, 1929). In the latter, however, adhesion is common and a tissue-like growth results.

Since sclerotia form in abundance on all hyphae the outline and position of the main radii are conspicuous. The accumulation of sclerotia on the main radii naturally is greater than on the single filaments because of the large number of sclerotia-producing hyphae—hence the dense linear masses of sclerotia.

Secondary radii consist of individual filaments and from these, branches grow horizontally in every direction, filling in all space on the substrate. The aerial hyphae in later stages apparently tend to contract, for the hyphae lose the typically gnarled appearance characteristic of fungous hyphae and become quite straight. They appear to be stretched, due perhaps to contraction of the hyphae to which they have become attached.

DISCUSSION

A fairly homogeneous medium is, as de Bary stated, necessary for the production of the well known ringed patterns. In such a medium the hyphae grow at a uniform rate and in radial directions from the spore as a center (Hein, 1927, 1928a, 1928b) thus forming a circular patch whose perfection of outline is proportional to the homogeneity of the medium. Presumably all such hyphal cells are of the same age and equipotent, therefore fruiting should and does occur at the same time on each hypha after a certain period of growth, the duration of which depends, as is well

known, on particular environmental conditions. A ring of fruit will thus be produced. Reproduction is said to occur when a certain stage of what we crudely call maturity is reached. Maturity, as is well known, can be hastened or retarded or entirely suppressed in many higher and lower plants as well as in animals by altering certain environmental conditions.

So, too, as Klebs (1904) has stated, reproduction depends on environmental conditions which are very specific and restricted. The vegetative or the growth period which appears to be a necessary preliminary step to the reproductive stage for any organism, has far less restricted environmental demands than the latter and for this reason, according to Klebs, may often continue along with the reproductive.

The rhythmic manifestations which occur under constant external conditions, autonomic in Pfeffer's (1905) sense or without coöperation of external conditions (autonomic according to Driesch) or which are not coincident with periodic environmental stimuli are of special interest.

The periodic external environmental factors associated with the zonal character of fungus growth are of varying importance in the case of the different species and are only indirectly concerned with zonation. According to Munk (1912) they are not to be regarded as specific form-determining stimuli, but as indirect stimuli, autonomic factors according to Pfeffer (1902); that is, external factors which produce internal change. They all probably bring about the same periodic internal conditions which may initiate either fruit production, sclerotial production, or other growth forms. According to Klebs (1904) the internal conditions which initiate fruiting may be the concentration of organic "stuffs," especially carbohydrates and related substances in the cell sap and protoplasm, specific nutritional conditions due to special thermally induced "Diffusionsverhältnisse" or increased transpiration caused by light or temperature either of which might bring about the concentration of plastic "stuffs."

That the concentric rings of fruiting structures, sclerotia, or of dense hyphal aggregates as in *Ascochyta Chrysanthemi* (1909) may be a response to Liesegang phenomena in the substrate is suggested by the similarity in appearance of the two kinds of rings. In both the Liesegang rings and in fungous rings there is in the beginning a patch, circular in outline, followed by rings which are close together in the early stages and then become increasingly wider apart away from the center outward. The inner margin of each circle in both the chemical rings and fungous rings is sharply defined while the outer margin is diffused and irregular.

Liesegang phenomena as explained by Ostwald (1899) depend on the relation between supersaturated solutions in which he distinguishes metastable solutions, those which apparently do not form the solid phase after a longer period, and labile solutions, those which under similar circumstances form the solid phase in a very short time. According to Ostwald:

Metastabile Lösungen zeigen den labilen aus den gleichen Stoffen gegenüber immer die kleinere Konzentration. Durch Vermehrung der Konzentration geht also eine metastabile Lösung in den labilen Zustand über. Die Konzentration, bei welcher der Uebergang erfolgt, heisst die metastabile Grenze." . . . "Der Niederschlag erfolgt aber nicht sofort, sondern erst, nachdem die stabile Grenze überschritten ist. Dies geschieht natürlich gleichzeitig in einem Kreise, der mit dem Tropfenkreise konzentrisch ist. Um den entstandenen Niederschlag lagert sich das Silberchromat, in bezug auf welches die Umgebung des Ringes übersättigt ist und verstärkt ihn; dies dauert so lange, bis das lösliche Chromat aus der Nähe entfernt, in den Niederschlag gegangen ist. Alsdann wandert das Silbersalz über den Ring hinaus, übersättigt ein neues, ferner liegendes, kreisförmiges Gebiet, und der gleiche Vorgang wiederholt sich. Da die Silberlösung beim Weiterdiffundieren immer verdünnter wird die kritische Konzentration, bei welcher die Ausscheidung beginnt, immer später erreicht, und der neue Ring entsteht erst in einem weiteren Abstand, als der zwischen seinen Vorgängern betrug.

A process similar to this which takes place in Liesegang's gelatin chromate plates (Küster, 1912) may take place in the agar substrate and the rings so formed may provide specific conditions which initiate either fruiting or dense aggregation of hyphae in the fungi.

I am of the opinion that the concentricity of fruiting rings or alternate dense and less dense hyphal growths under constant environmental conditions may be an expression of conditions analogous to those causing Liesegang phenomena. Such conditions may be present or develop either in the substrate or perhaps even in the hyphae themselves.

In cases where the rings are coincident with periodically recurring conditions such as diurnal light or temperature fluctuations, satisfying explanations and theories such as those given by Munk (1912), Milburn (1904), Molz (1907), Reidemeister (1908), and others may be given, but where no external periodic factors are involved the explanations so far given are inadequate. According to Munk's account, a certain degree of starvation or exhaustion of a particular food substance, especially carbohydrate, initiates conidial production. Such a stage will always be present somewhere in the zone surrounding the first ring and since it is obvious that there would be a gradual transition from the starved part of the substrate immediately beyond the area of conidial production to the uninvaded surrounding area, conidial production would occur in a centrifugally expanding circle. When the exhaustion of food substances is complete, starvation will prevent further growth. From the center outward we should then expect an ever widening uniform area of dead fungus. Munk's explanation could be applied if we were dealing with a single gradually widening ring as in the fairy rings of fleshy fungi. But his explanation, it seems to me, as do all others, fails to account adequately for the *alternation* of vegetative and fruiting rings.

Familiar examples of the diversity and widespread occurrence of rhythmically recurring periodic phenomena are the cyclic arrangement of floral and leaf organs, the striped pigmentation in such leaves as those of *Eulalia*

japonica, *Pinus Thunbergii* (*oculus draconis*), or *Haworthia fasciata*, the zonation in the molluscan shell, the striped and dotted patterns in feathers of birds, the wings of butterflies, the hair of animals, as well as zonal precipitates on the walls of glass cylinders from which water containing salts in solution has evaporated, the annual rings of woody plants, patterns of fish scales, bones and otoliths of fishes, mammalian bones, the chemically produced Liesegang rings, fairy rings, the striped patterns of agate, the banded structure of ice, quartz, agate, onyx, etc. The similarities in appearance of widely separated kinds of rhythmic periodic phenomena, as for example the green and white concentric bands shown in an apical view of *Pinus Thunbergii* (Küster, 1912, fig. 7) and the periodic rings of fruiting bodies in fungus cultures when compared with the chemically produced Liesegang rings suggest the possibility of a common explanation. Many kinds of periodic phenomena have been adequately explained as associated with particular environmental stimuli. In most cases, however, the periodic phenomena appear to be most intimately connected with a complex of both internal and external factors.

In experimental biology there is an increasing tendency to take account of the physical laws which in many cases determine the form and symmetry of organisms. The principle of minimal areas caused by surface tension as a form-determining factor has long been known. Among recent studies are those of Thompson (1917), Harper (1916, 1918, 1926, 1929), and Lewis (1923). This principle modified by contact, pressure, and other physical factors is certainly of great importance in the control of cell form and the symmetry of cell colonies and presumably also the form and symmetry of tissues and whole organisms.

One must be impressed by the importance of such simple mechanical stimuli as control the form changes which take place in *Dictyostelium* (Harper, 1926) and *Pediastrum* (Harper, 1918). Organic form and symmetry can be more effectively explained by the interaction of mechanical and chemical forces than by referring it to a mysterious something in the gene the inconstancy of which is shown by the great variations environmentally obtained in fungus cultures in such cases as are reported by Stevens and Hall (1909).

Symmetry was recognized as an aesthetic principle by the ancients and its importance in art is everywhere known. The beautiful many-times-figured radial and zonal patterns in fungi owe their aesthetic value to their symmetry. The widespread application of this principle in science generally has been most effectively pointed out by Jaeger (1920). In the structure of atoms in the molecule, the forms of crystals, the arrangement of leaves and branches, of floral organs, and of organic cells; everywhere the principle of symmetry finds expression.

That radial growth may be due to morphaesthesia has been earlier pointed out (Noll, 1900; Hein, 1928). Symmetry may be thought of as

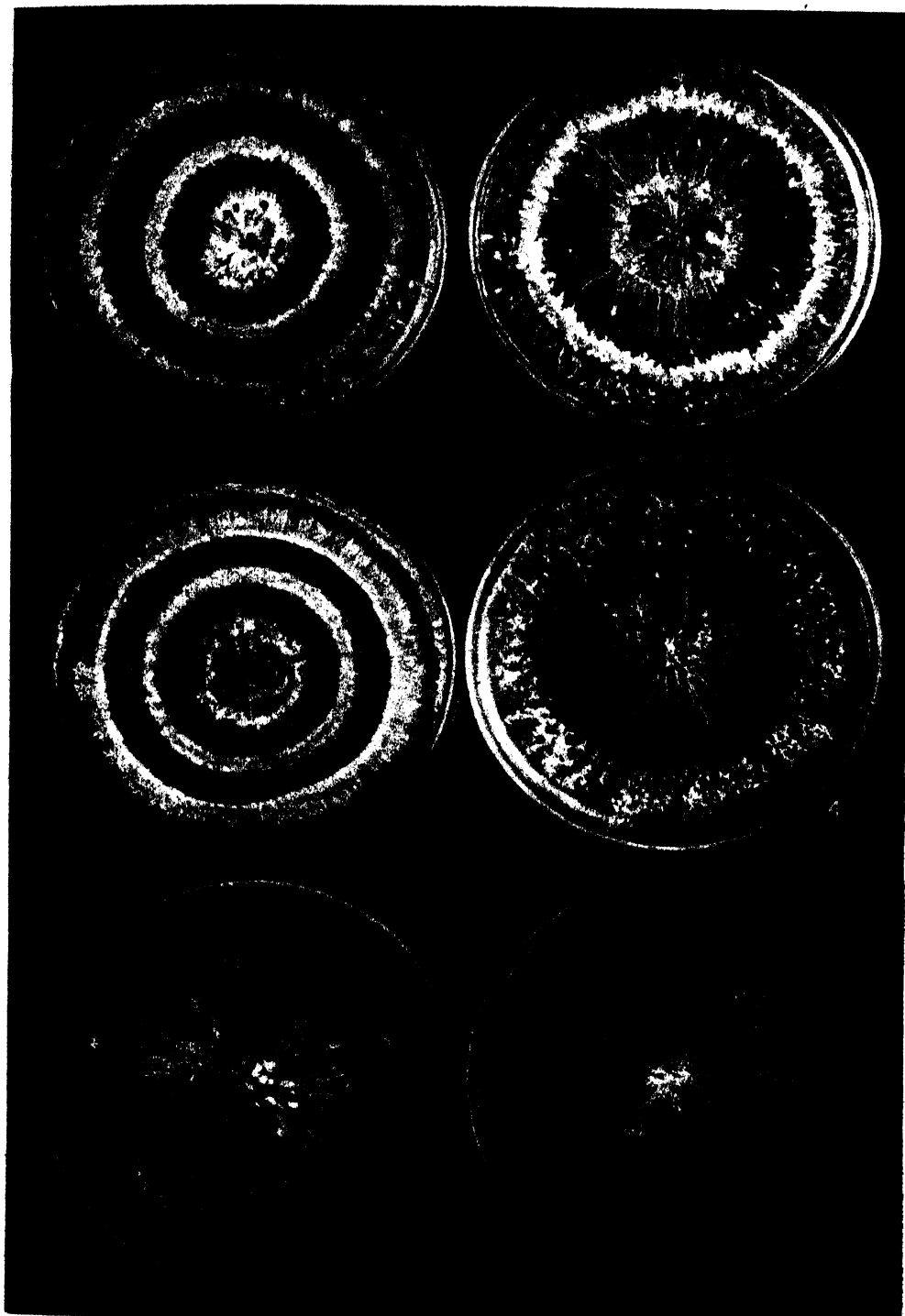
an expression of morphaesthesia and since symmetry in organisms has often been shown to be the result of surface tensions (Thompson, 1917) or form tensions (Küster, 1913) all symmetry relations may possibly be traced to this force. Millis (1918), in pointing out the importance of "the simple geometrical principle," raises the question whether "all life, growth, repair, decay, and dissolution: even all mind, intelligence, emotion, and all reasoning and thought" may not be intimately related to it.

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EXPLANATION OF PLATE VIII

- FIG. 1. Concentric rings of sclerotia grown in prune agar (25 gr. in 1000 cc. water).
- FIG. 2. The same fungus in less concentrated prune agar (20 gr. in 1000 cc. water).
- FIG. 3. Similar to figure 1 but in large 2 × 200 mm. culture dish.
- FIG. 4. Lima bean agar. Only one irregular ring is produced.
- FIG. 5. Corn meal agar. Irregular growth with no rings.
- FIG. 6. Nutrient agar. Dendritic branching and no rings.

THE CHROMOSOMES OF SOME DIOECIOUS ANGIOSPERMS

RUTH H. LINDSAY

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INTRODUCTION

The cytological investigations reported in the present paper were undertaken with the purpose of determining the exact circumstances of chromosome distribution in the division of the pollen-mother-cell nuclei of certain dioecious angiosperms. Attention has been focused on the stages of the heterotypic division in which the "homologous" chromosomes pair and separate, in order to detect any morphological difference between members of a pair that might be correlated with the usually dioecious condition of these species. No good evidence of such a difference has been found in *Bryonia dioica* L., *Clematis virginiana* L., *Smilax herbacea* L., *Menispermum canadense* L., or *Carica papaya* L., while the conspicuous heterochromosome pair of *Lychnis alba* Mill. (*Melandrium album* L.), already described by investigators in England, Sweden, and Germany, has been identified in material collected in the neighborhood of Madison, Wisconsin.

A preliminary report has already been made of some of the results of the present work (Lindsay, 1929).

RELATION OF CHROMOSOMES TO DIOECISM IN PLANTS

The subject of the factors determining sexual differentiation in dioecious organisms has been of great interest to biologists for many years. Experimental work on both plants and animals had already convinced some investigators that the sex of an individual is largely determined by some internal mechanism at the time of fertilization, when this conclusion received considerable support from cytological investigations of certain insects. A visible difference was found between the chromosome complements of males and females, and the origin of this difference was traced to the time of fertilization. In *Lygaeus*, to cite a well-known example, the female possesses a complement of equal pairs of chromosomes. The male, on the other hand, possesses a pair of chromosomes that differ in size, the larger member being of the same size as that of each member of the corresponding equal pair in the female. The separation of this so-called XY pair in the maturation divisions results in two classes of spermatozoa. An egg fertilized by a spermatozoon containing the larger member (X) of the unequal pair develops into a female; an egg fertilized by a spermatozoon containing the smaller member (Y) develops into a male. These hetero-

chromosomes in the male and the corresponding equal pair in the female have therefore been called "sex chromosomes," and it is assumed that they carry the genetic factors which, balanced with the normal complement of factors borne in the autosomes, determine the sex of the individual. A similar mechanism, and others differing only in that the smaller member of the pair is absent or that the larger member of the pair is multiple, have been reported for many other classes of animals. For one and perhaps two orders of insects, and for birds, the female has been found to contain the unequal pair, the spermatozoa in these cases being morphologically alike and the eggs of two kinds.

Following the discovery of a visible sex-determining mechanism in animals, cytological investigations were undertaken on various dioecious plants. Since 1917, a chromosome difference has been reported between the male and female gametophytes of 8 out of 16 species of dioecious hepatics studied cytologically, and between the staminate and carpellate plants of 43 out of 66 species of dioecious angiosperms similarly studied. So far it is only in these two groups of plants that heterochromosomes presumably related to sex-determination have been observed. The following tables give lists of dioecious hepatics and angiosperms in which the chromosome complement has been reported.

HEPATICS

Heterochromosomes present

| Species | Investigator |
|---|--|
| SPHAEROCARPALES | |
| <i>Sphaerocarpos Donnellii</i> | Allen, 1917, 1919; Lorbeer, 1927 |
| <i>Sphaerocarpos texanus</i> | Schacke, 1919; Lorbeer, 1927 |
| <i>Sphaerocarpos terrestris</i> | Lorbeer, 1927 |
| <i>Riella helicophylla</i> | Lorbeer, 1927 |
| JUNGERMANNIALES | |
| <i>Pellia Fabbroniana</i> | Heitz, 1928; (Lorbeer, 1927) |
| <i>Pellia Neesiana</i> | Showalter, 1927, 1928; (Lorbeer, 1927) |
| <i>Moerckia hibernica</i> (?) | Heitz, 1928 |
| <i>Makinoa crispata</i> (?) | Heitz, 1928 |

Heterochromosomes absent

| | |
|---|--------------------|
| MARCHANTIALES | |
| <i>Riccia Bischoffii</i> | Lorbeer, 1927 |
| <i>Riccia Curtisii</i> | McAllister, 1928 |
| <i>Marchantia polymorpha</i> | Lorbeer, 1927 |
| (<i>Marchantia</i> sp.) | Strasburger, 1909) |
| <i>Conocephalum conicum</i> | Showalter, 1921 |
| SPHAEROCARPALES | |
| (<i>Sphaerocarpos</i> sp. (?)) | Strasburger, 1909) |
| JUNGERMANNIALES | |
| <i>Riccardia pinguis</i> | Showalter, 1923 |
| (= <i>Aneura pinguis</i>) | Lorbeer, 1927 |
| <i>Blasia pusilla</i> | Lorbeer, 1927 |
| <i>Diplophyllum albicans</i> | Lorbeer, 1927 |
| <i>Scapania nemorosa</i> | Lorbeer, 1927 |

ANGIOSPERMS

Heterochromosomes present

| Species | Stage Studied | Investigator |
|--|--|---|
| HYDROCHARITACEAE | | |
| <i>Elodea gigantea</i> | meiosis in ♂ | Santos, 1923 |
| <i>Elodea canadensis</i> | { mitosis in ♂ and ♀ meiosis in ♂ | Santos, 1924 |
| <i>Hydrilla verticillata</i> | { mitosis in ♂ and ♀ meiosis in ♂ | Sinotô and Kiyohara, 1928 |
| PALMACEAE | | |
| <i>Trachycarpus excelsus</i> * | meiosis in ♂ | Sinotô, 1928 |
| DIOSCOREACEAE | | |
| <i>Dioscorea sinuata</i> * | meiosis in ♂ | Meurman, 1925 |
| SALICACEAE | | |
| <i>Populus tremula</i> | { mitosis in ♂ and ♀ meiosis in ♂ | Blackburn and Harrison, 1922, 1924 |
| <i>Populus trichocarpa</i> | meiosis in ♂ | Meurman, 1925 |
| <i>Populus balsamifera</i> | meiosis in ♂ | Meurman, 1925 |
| <i>Populus Simoni</i> | meiosis in ♂ | Meurman, 1925 |
| <i>Populus tremuloides</i> | meiosis in ♂ | Erlanson and Hermann, 1927 |
| <i>Populus serotina</i> | meiosis in ♂ | Blackburn, 1929 |
| <i>Populus Eugenei</i> | meiosis in ♂ | Blackburn, 1929 |
| <i>Populus generosa</i> | meiosis in ♂ | Blackburn, 1929 |
| <i>Salix viminalis</i> | meiosis in ♂ | Blackburn and Harrison, 1924; Sinotô, 1928 |
| <i>Salix cinerea</i> | meiosis in ♂ | Harrison, 1926 |
| <i>Salix lucida</i> | meiosis in ♂ | Harrison, 1926 |
| <i>Salix aurita</i> | meiosis in ♂ | Harrison, 1926 |
| <i>Salix Andersoniana</i> | meiosis in ♂ | Harrison, 1926 |
| <i>Salix leucopithecia</i> | meiosis in ♂ | Sinotô, 1928 |
| <i>Salix sachalinensis</i> | meiosis in ♂ | Sinotô, 1928 |
| <i>Salix melanostachys</i> | meiosis in ♂ | Sinotô, 1928 |
| <i>Salix japonica</i> | meiosis in ♂ | Sinotô, 1928 |
| URTICACEAE | | |
| <i>Humulus Lupulus</i> | meiosis in ♂ | Winge, 1923; Sinotô, 1929 |
| <i>Humulus japonicus</i> | meiosis in ♂ | Winge, 1923; Kihara, 1928 |
| <i>Cannabis sativa</i> | meiosis in ♂ | Hirata, 1924; Sinotô, 1928 |
| <i>Urtica dioica</i> * | meiosis in ♂ | Meurman, 1925 |
| <i>Morus bombycis</i> | meiosis in ♂ | Sinotô, 1928 |
| <i>Cudrania triloba</i> * | meiosis in ♂ | Sinotô, 1928 |
| POLYGONACEAE | | |
| <i>Rumex Acetosa</i> | { mitosis in ♂ and ♀ meiosis in ♂ | { Kihara and Ono, 1923, 1925 Sinotô, 1924 |
| <i>Rumex thyrsiflorus</i> | meiosis in ♂ | Meurman, 1925 |
| <i>Rumex Acetosella</i> | meiosis in ♂ | Meurman, 1925 |
| <i>Rumex arifolius</i> | meiosis in ♂ | Kihara and Ono, 1926 |
| <i>Rumex nivalis</i> | meiosis in ♂ | Kihara and Ono, 1926 |
| CARYOPHYLLACEAE | | |
| <i>Lychnis alba</i> | meiosis in ♂ and ♀ meiosis in ♂ meiosis in ♀ | Blackburn, 1923; Heitz, 1925 Winge, 1923; Lindsay, 1929 Meurman, 1925 |

* Not certain.

| Species | Stage Studied | Investigator |
|---|--|--|
| <i>Lychnis dioica</i> | meiosis in ♂ and ♀ | Blackburn, 1925; Heitz, 1925 |
| | meiosis in ♀ | Meurman, 1925 |
| <i>Lychnis alba</i> × <i>dioica</i> | meiosis in ♂ and ♀ | Blackburn, 1924 |
| <i>Lychnis glutinosum</i> | meiosis in ♂ and ♀ | Blackburn, 1928 |
| <i>Lychnis divaricatum</i> | meiosis in ♂ and ♀ | Blackburn, 1928 |
| <i>Silene Otites</i> | meiosis in ♂ and ♀ | Blackburn, 1928 |
| EUPHORBIACEAE | | |
| <i>Daphniphyllum macropodum</i> | meiosis in ♂ | Sinotô, 1928 |
| EMPETRACEAE | | |
| <i>Empetrum nigrum</i> | meiosis in ♂ | Hagerup, 1927 |
| DATISCEAE | | |
| <i>Datisca cannabina</i> | meiosis in ♂ | Sinotô, 1928 |
| VALERIANACEAE | | |
| <i>Valeriana dioica</i> | meiosis in ♂ | Meurman, 1925 |
| CUCURBITACEAE | | |
| <i>Trichosanthes japonica</i> | meiosis in ♂ | Sinotô, 1928 |
| <i>Heterochromosomes absent</i> | | |
| NAJADACEAE | | |
| <i>Najas marina</i> | mitosis in ♂ and ♀ | Winge, 1927 |
| ALISMACEAE | | |
| <i>Sagittaria montevidensis</i> | mitosis in ♂ and ♀ | Sykes, 1909 |
| HYDROCHARITACEAE | | |
| <i>Hydrocharis Morsus-ranae</i> | mitosis in ♂ and ♀ | Sykes, 1909 |
| <i>Vallisneria spiralis</i> | { mitosis in ♂ and ♀ meiosis in ♂ | { Winge, 1927 Jørgensen, 1927 |
| <i>Vallisneria gigantea</i> | mitosis in ♂ and ♀ | Jørgensen, 1927 |
| LILIACEAE | | |
| <i>Smilax herbacea</i> | meiosis in ♂ and ♀ | Elkins, 1914 |
| | meiosis in ♂ | Humphrey, 1914; Lindsay, 1929 |
| DIOSCOREACEAE | | |
| <i>Dioscorea caucasica</i> | meiosis in ♂ | Meurman, 1925 |
| <i>Dioscorea</i> sp. | meiosis in ♂ | Strasburger, 1909 |
| <i>Tamus communis</i> | meiosis in ♂ | Meurman, 1925 |
| MYRICACEAE | | |
| <i>Myrica rubra</i> | meiosis in ♂ | Sugiura, 1927 |
| URTICACEAE | | |
| <i>Cannabis sativa</i> | { meiosis in ♂ mitosis in ♂ and ♀ meiosis in ♂ | { Strasburger, 1909, 1910 McPhee, 1924 |
| POLYGONACEAE | | |
| <i>Rumex Acetosella</i> | meiosis in ♂ | Kihara, 1927 |
| CHENOPODIACEAE | | |
| <i>Spinacia oleracea</i> | meiosis in ♂ | Strasburger, 1909, 1910 Winge, 1923 Maede and Katô, 1929 |
| RANUNCULACEAE | | |
| <i>Thalictrum purpurascens</i> | meiosis in ♂ | Overton, 1909 |
| <i>Clematis virginiana</i> | meiosis in ♂ | Lindsay, 1929 |
| MENISPERMACEAE | | |
| <i>Menispermum canadense</i> | meiosis in ♂ | Lindsay, 1929 |
| SAXIFRAGACEAE | | |
| <i>Ribes alpinum</i> | meiosis in ♂ | Meurman, 1925 |

| Species | Stage Studied | Investigator |
|---------------------------------------|--------------------------------------|---|
| <i>Ribes saxatile</i> | meiosis in ♂ | Meurman, 1928 |
| <i>Ribes orientale</i> | meiosis in ♀ | Meurman, 1928 |
| ROSACEAE | | |
| <i>Fragaria elatior</i> | meiosis in ♂ and ♀ | Kihara, 1929 |
| EUPHORBIACEAE | | |
| <i>Mercurialis annua</i> | { meiosis in ♂ mitosis in ♂ and ♀ | Strasburger, 1909, 1910 |
| | meiosis in ♂ | Yampolsky, 1925 |
| <i>Mercurialis perennis</i> | mitosis in ♂ and ♀ | Sykes, 1909 |
| ACERACEAE | | |
| <i>Acer Negundo</i> | meiosis in ♂ | Darling, 1909; Mottier, 1914; Taylor, 1920 |
| CARICACEAE | | |
| <i>Carica papaya</i> | meiosis in ♂ | Meurman, 1925; Sugiura, 1927 |
| CORNACEAE | | |
| <i>Aucuba japonica</i> | meiosis in ♂ | Sugiura, 1927; Meurman, 1929 |
| CUCURBITACEAE | | |
| <i>Bryonia dioica</i> | meiosis in ♂ | Strasburger, 1909, 1910; Lindsay, 1929 |
| | mitosis in ♂ and ♀ | Sykes, 1909 |
| | meiosis in ♂ and ♀ | Meurman, 1925 |

In a similar list of hepatics, Kihara (1929b) includes *Riccia Bischoffii* as having been reported by Lorbeer in 1927 to have an *X*-chromosome in the female and a *Y*-chromosome in the male. Lorbeer's statement, however, is to the effect that in metaphase stages of the heterotypic division in *R. Bischoffii* one pair of chromosomes, apparently equal in size, is conspicuous because its members separate earlier than those of the 7 other pairs, a behavior similar to that of the *XY* pairs in *Sphaerocarpos* and *Riella*. Since no inequality between the members of the precocious pair in *Riccia* was observed, there seems to be no justification for calling it an *XY* pair. In the case of *Moerckia hibernica* and *Makinoa crispata* the evidence is not clear as to a chromosome difference between the two sexes, since Heitz (1928) based his conclusion on a study of the symmetry of chromosomes in the female gametophyte only, as compared with the chromosomes of *Pellia Fabbrianiana*, in which both sexes had been studied cytologically and in which the presence of an *X*- and *Y*-chromosome was reported.

In all hepatics in which a chromosome difference between the sexes has been observed, the female gametophyte is characterized by a large *X*-chromosome, the male by a smaller *Y*-chromosome. These differential chromosomes may justly be called "sex chromosomes," with the understanding that this term does not necessarily designate these chromosomes as the sole bearers of sex-influencing factors. Experimental evidence indicates that sex in the gametophytic generation of dioecious bryophytes is fixed and unalterable, and, until external influences are found that will change this condition, genetic factors may be assumed to play the leading

rôle in sex-determination. The fact that visible chromosome differences are not found in all dioecious hepatics may indicate only that the fundamental difference between sex chromosomes is qualitative rather than quantitative, and therefore does not necessarily result in visible differences. Evidence as to the effect of the presence of both sex chromosomes in the gametophytic generation is conflicting. Lorbeer (1927) reported a diploid gametophyte of *Sphaerocarpos Donnellii* which was purely female, while Showalter (1928) described a hermaphroditic diploid gametophyte of *Pellia Neesiana*. The XY pair was identified in the diploid *Sphaerocarpos* thallus, but not with certainty in the hermaphroditic *Pellia*.

In dioecious hepatics it is a haploid generation, with a single sex chromosome, that exhibits the characters of one or the other sex, while the diploid sporophytic generation, containing the unequal pair of sex chromosomes, is non-sexual. In dioecious angiosperms, on the other hand, sexual differentiation is exhibited also by the diploid generation, which possesses either an equal or unequal pair or group of sex chromosomes. This latter condition is similar to that found in animals, and it is probable that the relation of the sex chromosomes to sexual differentiation is very similar in these two groups of diploid organisms.

Of the angiosperms listed, two species, *Cannabis sativa* and *Rumex Acetosella*, have been the subjects of conflicting reports, and are for the present placed in both lists. So far, heterochromosome pairs or groups have been found only in the staminate plants of dioecious angiosperms. This finding is in accord with the hypothesis, held in general by those who have experimented with such plants, that the staminate plant is heterozygous, producing pollen grains (and ultimately male gametes) of two classes with respect to their sex potentialities. It is possible that there may be some exceptions to this rule, although no conclusive evidence of an exception has yet been reported. Following some experiments by Correns on *Fragaria elatior*, the results of which suggested that the carpellate plant might be heterogametic, Kihara (1926) studied the chromosomes of both staminate and carpellate plants of this species and reported what might be considered an unequal pair of sex chromosomes in the embryo-sac mother cell. Further study, however, convinced him that, although these two chromosomes might differ physiologically, there was no real difference in size between them, and that consequently *F. elatior* should be included in the list of dioecious angiosperms in which no heterochromosomes are visible (Kihara, 1929b).

The heterochromosomes found in angiosperms are of three types. In the majority of cases they are of the so-called XY type, in which case the chromosome formula for the staminate plant is $2n + X + Y$, with male gametes $n + X$ and $n + Y$; that for the carpellate plant $2n + 2X$, with eggs all $n + X$. A second type includes the tripartite and tetrapartite heterochromosome groups. In *Rumex Acetosa*, *R. thyrsiflorus*, *R. arifolius*,

R. nivalis, and *Humulus japonicus* the staminate plants have the chromosome formula $2n + Y_1 + X + Y_2$, with gametes $n + X$ and $n + Y_1 + Y_2$; the formula for the carpellate plants is $2n + 2X$. The tripartite group Y_1XY_2 separates in such a way on the heterotypic spindle that the two end chromosomes (Y_1Y_2) go to one pole, the middle chromosome (X) to the other. Meurman (1925) reported a similar group of three in *Rumex Acetosella*, but stated that this group separates in such a way that the middle and one end chromosome pass to one pole while the other end chromosome passes to the other pole. Kihara (1927) thinks that this tripartite complex reported by Meurman may either have been misinterpreted as such or may have become a tripartite complex through fusion end to end of two of the chromosomes of a tetrapartite group, and that it cannot be stated definitely that *R. Acetosella* has a sex-chromosome complex. Winge (1923) reported an unequal pair for both *Humulus Lupulus* and *H. japonicus*. The tripartite group described above for *H. japonicus* was reported by Kihara (1928), and a tetrapartite group has been reported for *H. Lupulus* by Sinotô (1929). This group consists of four chromosomes, of which the two middle ones, X_2Y_1 , are equal in size and larger than the two end ones, X_1Y_2 , which differ in size from each other. These four chromosomes are arranged end to end, $X_1Y_1X_2Y_2$, and separate in such a way on the heterotypic spindle that X_1 and X_2 go to one pole and Y_1 and Y_2 to the other. The carpellate plant is assumed to have an $X_1X_2X_2X_1$ group. Sex-chromosome complexes of this second type have no parallel in any group of animals, although instances are found among animals of a multiple X -element. The third type, the so-called XO type, has been reported as probable for *Dioscorea sinuata* by Meurman (1925). In this case the staminate plant, with the formula $2n + X$, has one less chromosome than the carpellate plant, whose formula would be $2n + 2X$. The same chromosome complement was reported for *Vallisneria spiralis* by Winge (1923), but the description was later shown to be incorrect by Jørgensen (1927) and Winge (1927).

The evidence from polyploid species is conflicting. Harrison (1926) reported the presence of heterochromosomes in three tetraploid and one hexaploid species of *Salix*. He concluded that simple chromosome doubling could not account for the origin of polyploidy in these species, since in that event one would expect to find the heterochromosomes also doubled, whereas careful examination of diploid, tetraploid, and hexaploid species showed only one unequal pair present in each case. Hagerup (1927) reported the presence of an XY pair in the staminate plant of *Empetrum nigrum*, a dioecious species, and two XY pairs in *E. hermaphroditum*, a tetraploid hermaphroditic species. Ono (1928) reported a triploid carpellate plant of *Rumex Acetosa* with three X -chromosomes, and triploid intersexual plants with two X - and two Y -chromosomes, and Ono and Shimotomai (1928) described triploid and tetraploid intersexual plants of *R. Acetosa*.

with the probable chromosome formula $3n + 2X + 2Y$ and $4n + 3X + 2Y$. Thus, in *Salix*, it appears that dioecism is not interfered with by polyploidy, although the XY pair remains unduplicated and the balance between sex chromosomes and autosomes is thereby upset. In *Empetrum* the XY pair is duplicated along with the autosomes and hermaphroditism results, although the balance between sex chromosomes and autosomes remains the same as in the diploid staminate plant. In *Rumex*, when polyploidy preserves the balance between sex chromosomes and autosomes the plant is unisexual, when the balance is upset the result is hermaphroditism.

Sex in dioecious angiosperms is by no means unalterable, and the possibility of sex-reversal cannot be limited to those species in which no sex-determining mechanism is visible. *Cannabis sativa*, for which an XY chromosome pair has been reported by two investigators, has been the object of numerous successful experiments in sex-reversal, and in the case of *Lychnis dioica*, which affords the classic example of an XY pair, hermaphrodites with the normal chromosome complement of the staminate plant are known to occur. In view of these facts and of the contradictory evidence from polyploid species, it is clear that the relation of sex chromosomes to sexual differentiation in dioecious angiosperms cannot be a simple one. The use of the term "sex chromosomes" seems justified, however, if it is used with the understanding that the chromosomes so designated are assumed to carry the factors which, subject to the interaction of other internal and external factors, swing the balance in favor of the expression of one or the other sex in a plant developing under ordinary environmental conditions.

In many of the dioecious species investigated, the staminate plant alone has been examined. Where an unequal pair of chromosomes has been found in these cases, it is safer to refer to them as heterochromosomes rather than as sex chromosomes until the carpellate plant has also been examined, although their connection with sex-determination may appear highly probable. It is to be remembered, however, that the presence of heterochromosomes does not always indicate the existence of a sex-determining mechanism. The heteromorphic pairs of chromosomes found by Miss Carothers (1921) in an orthopteran segregate at random with respect to the sex chromosome and to each other, and clearly have no relation to sex. Preparations made in the botanical laboratory of the University of Wisconsin by Mr. D. C. Cooper of the pollen mother cells of *Bougainvillea spectabilis* show an unequal pair of chromosomes in various stages of separation on the heterotypic spindle. Since this is a hermaphroditic species, the unequal distribution of chromosome material can have no conceivable connection with sex differentiation.

MATERIALS AND METHODS

Staminate buds of *Smilax herbacea*, *Menispermum canadense*, and *Lychnis alba* were obtained during the summers of 1927 and 1928 from

plants growing wild in the vicinity of Oconomowoc and Madison, Wisconsin. The source of the buds of *Clematis virginiana* obtained in 1927 and 1928 was a vine that had been under cultivation in a private garden near Oconomowoc for more than ten years. Staminate buds of *Carica papaya* were obtained in December, 1928, from one tree growing in a semi-cultivated condition on the property of Mr. J. W. Hargrave in St. Petersburg, Florida. The staminate buds of *Bryonia dioica* used in the present study were from a lot of young staminate and carpellate flowers and buds fixed in October, 1910, by Professor C. E. Allen. The plants that supplied the material were grown from seed obtained of Haage and Schmidt, Erfurt.

The *Bryonia* material was fixed in Flemming's strong and medium solutions, and some of the material sectioned and stained by Mrs. Nellie Morey under Professor Allen's direction during the year 1910-11; the remainder of the material was prepared and studied by the present author during the year 1915-16. The observations recorded in this paper are the result of that study and of a re-examination of the slides during the past year.

A number of fixatives were used for all material except *Bryonia* and *Lychnis*, including Flemming's strong, medium, and weak solutions, formol-acetic-alcohol, and Carnoy's chloroform-acetic-alcohol. The two last-named gave uniformly the best results for chromosome study, with Carnoy's mixture slightly superior, and almost all later fixations, including all those of *Lychnis*, were made with these two fixatives. Some of the *Clematis* material of 1928 was fixed in Carnoy's mixture ten seconds to five minutes, followed by Flemming's strong solution, these fixations also giving excellent results and being especially valuable when followed by Newton's iodine-crystal violet stain. The customary methods of dehydrating, hardening, clearing with chloroform, and imbedding in paraffin were followed. Sections were cut from 4 to 15 microns in thickness, in order to obtain sections of parts of spindle figures for study of individual chromosomes, as well as complete nuclei in diakinesis, and entire spindles.

Flemming's triple stain and Heidenhain's iron-alum-haematoxylin were used, as a rule being alternated in each series of sections. Newton's iodine-crystal violet stain following the Carnoy-Flemming fixation proved especially valuable for the study of individual chromosomes in *Clematis*. This stain colors only the chromosomes, the remainder of the cell structures being practically colorless.

OBSERVATIONS AND DISCUSSION

Bryonia dioica

Bryonia dioica is one of the few dioecious angiosperms that have been the object of experimentation with reference to sex-determining factors. The well-known study by Correns (1907) of the sexes of crosses between *B. alba* and *B. dioica* convinced him that a separation of sex tendencies

takes place in the division of the pollen mother cells of the latter species, with the result that half the pollen grains carry the female tendency and half carry the male tendency. Bateson (1909), while confirming Correns' experimental results, explained them in a quite different way, his hypothesis postulating heterozygosis in the female of *B. dioica* and homozygosis in the male. On this assumption only one kind of pollen grain, so far as sex tendencies are concerned, would be formed. Strasburger (1900) was led by the failure of his attempts to induce the production of male flowers on shoots of a female plant of *B. dioica* to conclude that sex in dioecious angiosperms is already determined in the embryo. In 1910 he adopted the hypothesis, previously advanced by Noll, that the pollen grains of dioecious angiosperms all carry the male tendency, but that in one half of the pollen the male tendency is stronger than the female tendency carried by the egg, in the other half weaker.

Cytological examinations of *B. dioica* have not been reported in much detail. Strasburger (1910) spoke of the haploid number of chromosomes as 10 and said that he found no evidence of a cytological basis for sex-separation in the nuclear divisions in the pollen mother cell. Miss Sykes (1909) reported that the nuclei in the male and female plants of *B. dioica* are apparently identical as to the number and character of their chromosomes. Meurman (1925) studied the meiotic divisions in both staminate and carpellate plants and reported no unequal pair present. He agrees with Strasburger that the haploid number is 10.

The results of the present cytological study are in essential agreement with previous accounts, although some hitherto unreported details have been observed.

From the stage of diakinesis to the end of the heterotypic division, the chromosomes in the pollen mother cells are characteristically short and thick, most of them being about as broad as they are long. Plate IX, figure 1, shows one pair with attenuated ends, a condition never observed after all traces of the linin portions of the spireme have disappeared in the nucleus. In diakinesis (fig. 2) the two chromosomes of a pair lie parallel, and usually so loosely associated that the outline of each is very distinct. This parallel arrangement affords an excellent opportunity for comparing the size and shape of the two members of each pair, and it is obvious that any morphological difference would stand out clearly. In no case has an inequality been visible. A budding of the nucleolus is frequently noted at this time (fig. 2), but at no stage are the buds seen to be actually cut off, and there is no evidence of any connection between such budding and an increase in number of nucleoli.

There appears to be a time of fairly close association between the chromosomes of a pair at the end of diakinesis (fig. 3), when the nuclear membrane disappears and cytoplasmic fibers invade the nuclear cavity. At this stage some of the pairs appear to be indistinguishably merged, while others still show their double nature.

It is noteworthy that the chromosomes are at no time seen to be arranged in what might be called an equatorial plate. Apparently they begin to separate almost as soon as the fibers become attached to them, and by the time that the fibers are arranged in a bipolar spindle the separation of the chromosomes is well advanced (fig. 4). From this time to the end of the anaphases it is easy to count the chromosomes; a number of counts make it certain that the reduced number is 10, a conclusion in agreement with that of Strasburger (1910) and Meurman (1925). Figures 5, 6, and 8 each show all 10 pairs in side view on the spindle, and figures 14 and 15 give polar views of anaphase groups, each showing 10 split chromosomes.

In the metaphases the two chromosomes of each pair seem to separate with difficulty and often remain united for some time by a slender thread which may be pulled out to a considerable length (figs. 4, 10, 11). After the chromosomes are completely separated, they progress toward the respective poles in a sidewise, cornerwise, or edgewise position, apparently depending upon the way in which they happen to be attached to the fibers (fig. 13).

The so-called second longitudinal split is evident in some chromosomes at the time of their separation. The split is often to be seen in a pair that are not yet completely separated (figs. 8, 11, 12), and by the time that all the chromosomes are on their way to the poles the split is plainly visible in every case (fig. 13). It appears from an examination of metaphase figures that there is characteristically one chromosome pair that is precocious in the matter of splitting. In figures 7 and 9, which show the chromosomes of figures 6 and 8, respectively, spread out in a row, this split chromosome pair (*s*) is conspicuous in comparison with its fellows. A second pair in figure 9 also shows a split, but the separation of the two halves of each member is not so wide as in the precocious pair. In very lightly stained preparations showing metaphase figures, a less conspicuous split may be observed in several chromosomes on one spindle (figs. 11, 12), but these chromosomes can be readily distinguished from the single pair showing a wide separation of the two halves of each member (cf. figs. 5, 6, 11). The precocious pair has not been identified with certainty in later stages. Figures 14 and 15, which give polar views of anaphase chromosomes, show the longitudinal split almost as clearly as it appears in lateral view.

The spindle figure occupies a comparatively small area in the cell, its diameter being between one-third and one-fourth that of the cell, which is characteristically spherical at this stage (fig. 4).

Relatively few preparations showing the homoeotypic division were obtained, but in such figures as were found there is no indication of any irregularity in this division.

The fact that all the chromosome pairs may be distinguished on a single heterotypic spindle in lateral view, together with the clear outlines of paired chromosomes in diakinesis, makes possible the definite conclusion that in

Bryonia dioica there is no morphological difference recognizable by methods at present available between the two chromosomes of any pair in the pollen mother cells.

Clematis virginiana

So far as published results show, the only cytological study that has been made of any of the species of *Clematis* is that by Guignard (1885). He figures nuclear division in the endosperm cells of *C. recta* and states that the nuclear plate consists in general of 16 elements.

The pollen mother cells of *C. virginiana* offer excellent material for the study of chromosome morphology, and with *Bryonia dioica* afford the most convincing evidence in any of the plants studied of the equality of the members of each chromosome pair. The haploid number is 8, all bivalents being large and distinct, and it is possible to find side views of metaphase figures in which the 8 pairs can be readily distinguished (fig. 19).

The stages of diakinesis are not so favorable for observing the relative sizes of the two members of each pair, since besides forming V's and X's they also twist about each other in various shapes (fig. 16). In many cases definite constrictions appear at exactly corresponding regions in the two members of a pair (fig. 17), probably indicating the point of fiber-attachment in the future atelomitic chromosomes. A few cases were found in which chromosomes, apparently displaced by the knife in cutting, had been broken at the point of fiber-attachment, and the probability that this point is relatively weak is also indicated by the pulling out of this portion of the chromosome to a comparatively thin thread in some early anaphase figures (fig. 23c).

Instances are found of what is apparently a satellite attached to the end of one member of a pair (figs. 16, 17), but this is probably a bit of incompletely absorbed linin connective, and does not persist as a characteristic of any individual chromosome. In later stages no chromosomes are observed with satellites.

In metaphases and early anaphases it becomes apparent that 6 of the 8 pairs are characteristically atelomitic and that 2 are telomitic. Figures 24 and 25 show 7 and 8 pairs, respectively, from two different cells, the 2 telomitic pairs being included in each group. One of the atelomitic pairs is sometimes observed to have an apparently median fiber-attachment (fig. 23a), but in somewhat later stages of separation the chromosomes of all 6 atelomitic pairs show one free arm that is shorter than the arm still attached to, or just separating from, its partner (fig. 25). In later anaphases (figs. 27, 28) it is difficult to determine the exact point of fiber-attachment, owing to the various positions on the spindle of the separated V's, but more than one chromosome at this stage appears to have a median fiber-attachment (fig. 28). This condition suggests that the chromosome material is more or less elastic, and that when the tension on the long arm has been released by final separation, this arm may contract. Guignard

(1885) figures the somatic chromosomes in anaphases as U's, and states that they take this form as soon as the separation of the halves is completed. That the chromosome material is plastic is evidenced by the occasional pulling out of the attached arms of the separating chromosomes into an irregular thread (figs. 23*c-e*, 27).

Evidence of the heterogeneous structure of the chromosomes is given by their uneven outline, even in stages at which the forces causing the separation of chromosome pairs might be supposed to smooth out irregularities in a plastic substance. Further evidence on this point is given by some of the chromosomes in lightly stained preparations, in which two rows of more darkly staining areas are visible in a part at least of the body of the chromosome, these areas corresponding to the unevenness at the surface of the chromosome (figs. 22, 23*b*).

The splitting of the chromosomes for the homoeotypic division is evident in a very few cases as soon as the chromosomes begin to separate on the heterotypic spindle. The two rows of dark-staining bodies just referred to are usually separated by a much lighter area, indicating at least the double nature of the chromosome at this stage (figs. 22, 23*b*), and in some cases a definite split is seen (fig. 21). These cases are rare, however, most of the chromosome groups in metaphases and early anaphases giving no evidence whatsoever of a double nature. The split apparently occurs or is completed rather suddenly in the later anaphases. Figure 26 gives a polar view of an equatorial plate, in which the end view of each chromosome shows it to be still intact, while figures 29 and 30 each give a polar view of a retreating group in late anaphase showing 5 chromosomes with four free ends each, and 2 with two free ends each, the latter probably being the 2 telomitic chromosomes.

The spindle figure in the heterotypic division occupies a large part of the cell in comparison to the usual situation in *Bryonia* (cf. figs. 4 and 18). The diameter of the equatorial plate is more than one half that of the cell, and the cell characteristically gives an impression of being crowded with chromosomes.

In *Clematis*, as in *Bryonia*, the possibility that all the chromosome pairs of one nucleus may be seen in profile view in one section, or in two succeeding sections, makes the observer confident that no visible difference between the two chromosomes of any pair has been overlooked.

Smilax herbacea

The divisions in the pollen mother cells of *Smilax herbacea* have already been described by two investigators. Miss Humphrey (1914) reported 12 as the haploid number of chromosomes, and Miss Elkins (1914) reported 12 or 13, with no evidence of an unequal pair. The present study has shown 13 to be the correct number, that count having been made in numerous polar views of the heterotypic metaphases and anaphases, and in polar views of homoeotypic anaphases.

The chromosomes in diakinesis are very irregular in outline. Linin connective strands are still visible up to the time that the nuclear membrane becomes indistinct (Pl. X, fig. 31), such a strand sometimes giving the appearance of a satellite attached to one member of a pair as in *Clematis* (cf. Pl. IX, fig. 16, and Pl. X, fig. 32). Bivalents are occasionally found twisted in the form of an 8 (fig. 32), but the usual condition in diakinesis is a nearly parallel arrangement of the two chromosomes of a pair. No conclusion can be reached as to the relative size and shape of the two members of a pair at this stage, however, because of their uneven outline and their occasional apparent fusion. No stage has been found between late diakinesis and the equatorial plate, at which time the chromosomes have rounded up and lost all trace of linin strands. There is thus no evidence of a multipolar spindle, but this observation is in accord with Miss Humphrey's statement that the spindle is bipolar from the first.

No evidence has been found in the preparations studied of the so-called second longitudinal split during the metaphase stages, although Miss Elkins figures a distinct splitting of some of the chromosomes at this time. Most of the chromosomes are atelomitic (fig. 35), but the exact number of such chromosomes has not been determined, since in no case are all 13 pairs to be seen in profile view on one spindle. The bivalents in diakinesis offer no evidence on this point, being very uneven in outline and showing no regular constrictions. Some of the chromosomes in later anaphases are decidedly V-shaped, while some seem to be rod-shaped (figs. 40, 41). However, in polar views of very late anaphases all the chromosomes appear double, the two parts of each chromosome being in close approximation (fig. 42). It is possible that this double appearance is due to a splitting rather than to the approximation of the two ends of each chromosome, although a longitudinal splitting of V-shaped chromosomes at this stage should give polar views in which some of the chromosomes at least would show four free ends each. Possibly further study will discover such figures; for the present the question must be left open as to when the so-called secondary split becomes evident.

One pair of chromosomes, usually found on the periphery of the spindle, is distinctive in its manner of separating. The point of fiber-attachment is very near the end, so that, in separating, the two chromosomes present two relatively long free ends radiating out at right angles to the spindle, or appear as a thick V with the apex at right angles to the spindle (figs. 33, 34, 37). Figures 16 and 17 in Miss Elkins' paper show this pair. No mention is made of it in her discussion, although she states that one pair is frequently seen to separate earlier than the rest. These chromosomes with their radiating ends are usually distinct in polar views of the metaphase group (figs. 38, 39), but appear to fall in line with the other chromosomes as they are drawn to the poles.

While the positive evidence available in *Bryonia* and *Clematis* could

not be duplicated here, nevertheless the examination of at least two hundred anaphase groups, in each of which from 6 to 11 chromosome pairs could be seen in profile view on one spindle, makes it fairly certain that there is no visible difference between the chromosomes of any pair as they are separated on the heterotypic spindle.

It should be noted here that the separating chromosomes of a pair in the heterotypic division sometimes give an impression of inequality. Careful examination, however, always reveals the fact that this impression is due to the folding over of the free end of what is apparently the smaller member in the plane of observation, while the free end of the other member is seen in profile view (fig. 36a). The similarity of these figures to some of the published figures of unequal chromosome pairs, where no evidence aside from a size difference on the heterotypic spindle is offered, leads one to suspect that further study of some of these forms might be valuable. In *Smilax*, when both members of a pair are seen in profile, there is never any question of a difference in size.

Smilax, the only monocotyledon of the group studied, affords the only example of cell division preceding the homoeotypic division (figs. 44, 46). Miss Humphrey figures a distinct wall between the homoeotypic spindles, but Miss Elkins shows only the wall of the pollen mother cell surrounding the two spindles. The homoeotypic division involves the apparently equal splitting of chromosomes (fig. 43), and a count of chromosomes in polar view in a cell in which the spindles are parallel shows 13 chromosomes in each daughter cell (fig. 46).

On the whole, the evidence is fairly conclusive that the result of heterotypic and homoeotypic divisions in the pollen mother cells of *Smilax herbacea* is an equal distribution of chromosomes to the four microspores.

Menispermum canadense

The chromosomes of *Menispermum canadense* are small and fairly uniform in size, and so compactly arranged on the spindle that only in a very thin section containing but one half or less of the spindle can individual chromosome pairs be studied (fig. 55). The chromosomes in polar views of both heterotypic and homoeotypic metaphases are close together but very evenly spaced, and in every case in which the individual chromosomes are distinct the haploid number has been found to be 26 (Pl. XI, figs. 67-71).

The chromosomes in diakinesis are short, paired rather loosely, and never twisted about each other (Pl. X, fig. 52). Traces of linin connectives persist for some time (figs. 50, 51), but these have completely disappeared before the nuclear membrane becomes indistinct. The two chromosomes of a pair can usually be distinguished, but throughout the stages of diakinesis there are always several pairs in a nucleus that appear to be more or less fused. The appearance in one or two nuclei in late diakinesis of what is apparently a trivalent complex (fig. 52) has led to a careful examination

of more than a hundred nuclei at this stage to ascertain whether or not this might be a constant occurrence. No definite trivalents have been found, but both members of one or two pairs in a nucleus are often seen to be two-lobed and curved in such a way as to make a more or less four-lobed circle (fig. 51). What is apparently a group of three might conceivably be such a group in which the lobing of one member is not evident. An examination of nuclei in the early stages of diakinesis reveals many pairs with chromatin still strung out on the linin strands, and even distinct cases of lobing (fig. 49). When the chromosomes lie free in the cytoplasm just after the disappearance of the nuclear membrane, instances are found in which all pairs are fused with the exception of one or two, which retain their double appearance (fig. 54). It is possible that this is a constant characteristic of certain chromosomes, but the evidence is not sufficient to make it certain.

No evidence of a multipolar spindle has been found. The felted zone of cytoplasm around the chromosome group becomes transformed in some way into a bipolar spindle, with the chromosomes arranged in a compact equatorial plate (fig. 55).

Metaphase and early anaphase figures show as a rule a separation of equal chromosome pairs, but here again, in six cases out of about two hundred such figures examined, there has been observed what might be interpreted as a group of three chromosomes separating in the manner of the tripartite chromosome complex reported by Kihara and Ono (1923) for the staminate plant of *Rumex Acetosa*. Plate XI, figures 56 to 61, show all such instances found, and it is obvious that in each case the chromosome group in question could be interpreted either as the separation of a pair of lobed chromosomes, or as the separation of a pair showing a precocious split (cf. *Bryonia*, Pl. IX, figs. 6, 8). In any event, in no case can it be called a clear demonstration of a tripartite chromosome group. More positive evidence lies in the consistent count of 26 for the chromosomes seen in polar view of metaphase or early anaphase. In at least one cell in which the homoeotypic spindles were parallel and cut in cross section, 26 chromosomes were counted on each spindle (Pl. XI, fig. 71). On the whole, the relative infrequency of the observations of an apparent group of three, together with the consistent count of 26, justify no other present conclusion than that the usual procedure in the heterotypic division involves the separation of two similar chromosome groups. Owing to the large number of chromosomes and the failure so far to identify all 26 pairs in one nucleus or even in successive sections of a spindle, it is not possible to decide whether or not the lobing and curving observed in diakinesis and possibly in metaphase is a constant characteristic of one or two individual chromosome pairs.

The split for the homoeotypic division is not visible in the separating chromosomes, even in late anaphases. Various deviations from the ordinary history have been observed in the separation of the pairs in early heterotypic

anaphases. Occasionally a pair is seen to be separating with apparent difficulty (figs. 63, 65*a*, 66), or one of the pair may be drawn out into an irregular thread (fig. 65*b*). The apparent inequality of the pairs at the right in figures 64*a* and 65*a*, respectively, is possibly due to a displacement in cutting. The homoeotypic division figures show no inequalities in chromosome distribution.

In spite of the relatively infrequent observations of slight abnormalities and of the failure to identify all chromosome pairs in one cell, the conclusion seems justified for the present that the chromosomes of each segregating pair in the pollen mother cells of *Menispermum* are morphologically similar.

Carica papaya

Reyes (1925) reported that *Carica papaya* is normally dioecious, but may be widely variable as to sexual expression. The staminate flower always shows a rudimentary pistil, and the staminate plant may produce hermaphroditic or purely carpellate flowers. No case has ever been reported of sex-reversal in a carpellate plant, and the carpellate flower has not even a vestige of a stamen. The experiments carried out by Reyes resulted in failure to produce any sex-alteration in the carpellate plants, and in the production of but an incomplete and temporary sex-reversal in the staminate plants.

The pollen mother cells of *C. papaya* have been studied by two investigators. Meurman (1925) gave 9 as the haploid number of chromosomes and reported no unequal pair. Sugiura (1927) in a more detailed report confirmed Meurman's observations.

The division figures examined in the present study show an absolutely regular separation of equal pairs of small chromosomes on both heterotypic and homoeotypic spindles (figs. 78, 83). In spite of the small number and small size of the chromosomes, all the pairs have not been identified with certainty on one spindle, but counts made from polar views indicate that 9 is the haploid number, in accordance with the observations of previous authors.

The chromosomes in diakinesis are small and oval, and as a rule rather loosely associated. Members of a pair are frequently united by a delicate thread, possibly a remnant of the linin portion of the spireme (figs. 72-74). As in *Bryonia dioica*, a period of close association between the chromosomes of each pair appears to intervene between the disappearance of the nuclear membrane and the formation of the spindle (fig. 75). Sugiura (1927) reports the formation of a multipolar spindle, and some evidence of such a condition has been observed in the present study (figs. 75, 76).

It is noteworthy that here again, as in the case of *Bryonia*, the chromosomes are at no time seen to be arranged in what might be called an equatorial plate. By the time that the fibers are arranged in a bipolar spindle, most of the pairs are completely separated. The spindle figure occupies a

comparatively small area in the cell, and the chromosome pairs are widely spaced (figs. 77, 78, 80). There is no indication of an inequality between the two chromosomes of any pair, although their small size makes an observation of details of structure impossible. The splitting in preparation for the homoeotypic division was not observed, although Sugiura (1927) reports that it is visible in the resting nuclei during interkinesis.

The chromosomes appearing in the homoeotypic division are very small in comparison to those in the heterotypic division (figs. 82, 83), a fact also noted by Sugiura. Their division is apparently quite regular, and it is highly probable that the normal procedure in *Carica papaya* is an equal distribution of chromosomes in both of the divisions in the pollen mother cells.

Lychnis alba

The genus *Lychnis* is interesting because it is one of the clearest cases of a dioecious plant with a visible sex-determining mechanism, in which the occurrence of hermaphrodites has been reported. The production of stamens in flowers of carpellate plants of *L. dioica* when the plants are infected with *Ustilago violaceae* has been reported by several investigators (Strasburger, 1900; Shull, 1912). Shull (1909, 1911) and the Hertwigs (1922) have reported the occurrence, in pure-bred families of *Lychnis dioica*, of hermaphroditic individuals which behave like modified males in genetic experiments, and which (according to Bělař, 1925) possess the chromosome complement of the normal staminate plant.

An unequal pair of chromosomes in the staminate plants of four species of *Lychnis* has been reported by several observers. The genus has come to be the classic example of a dioecious angiosperm with a sex-chromosome pair, since the *XY* pair is so conspicuous and has been figured by so many investigators.

The pollen mother cells of *L. alba* have been observed in the present study chiefly for purposes of comparison with the other species examined. Division figures have been found exactly similar to those published by Miss Blackburn (1924) and others (figs. 84-88). The smaller member of the unequal pair is frequently folded over in the plane of observation as in the case of some of the chromosomes of *Smilax herbacea* referred to earlier (fig. 86), but in *Lychnis* both members of the pair may just as frequently be seen in profile view, in which case their size difference is unquestionable (figs. 84, 85). It is of some interest that this is the first report of the unequal pair in material collected in this country of any species of *Lychnis*.

SUMMARY

1. No evidence has been found of an unequal pair of chromosomes in the division of the pollen mother cells of *Bryonia dioica*, *Clematis virginiana*, *Smilax herbacea*, or *Carica papaya*.
2. Although there is some indication of a peculiar behavior on the part

of one or two chromosome pairs in *Menispermum canadense*, there is not sufficient evidence to prove this a constant characteristic. The conclusion seems justified that the heterotypic division in the pollen mother cells normally results in an equal distribution of chromosomes.

3. In *Lychnis alba* the large unequal pair of chromosomes in the pollen mother cells has been identified.

4. The haploid number of chromosomes is 10 in *Bryonia dioica*, 8 in *Clematis virginiana*, 13 in *Smilax herbacea*, 9 in *Carica papaya*, and 26 in *Menispermum canadense*.

5. One pair of chromosomes in *Bryonia* frequently shows a precocious splitting on the heterotypic spindle. This is a nearly or quite constant characteristic and usually distinguishes this chromosome pair from the others in metaphase and early anaphase stages.

6. One pair of chromosomes in *Smilax* may regularly be distinguished from the others on the heterotypic spindle by the manner of its separation. Its identification is impossible in later anaphases.

7. An occasional pair of chromosomes in *Smilax* gives an impression of inequality, due to the folding over of the free end of what is apparently the smaller member of the pair in the plane of observation. When both members are seen in profile, there is never any question of a difference in size.

8. In *Bryonia*, *Menispermum*, and *Carica* a period of close association of the paired chromosomes intervenes between the disappearance of the nuclear membrane and the formation of the spindle.

9. In *Clematis* the heterogeneous structure of the chromosomes is indicated by their uneven outlines and by rows of more darkly staining areas in the body of individual chromosomes.

10. *Smilax*, the only monocotyledon studied, affords the only instance of cell division between the heterotypic and homoeotypic divisions.

11. Great variation is noted in the six species examined in the relation of total chromosome material and spindle size to the amount of cytoplasm in a given cell.

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EXPLANATION OF PLATES

All figures were drawn with a 2 mm. apochromatic objective, at table level, with the aid of a camera lucida. Drawings of *Bryonia*, *Clematis*, and *Smilax* (figs. 1-46) were made with a Leitz compensating ocular no. 12, a magnification of about 2700 × being obtained. For drawings of *Menispermum*, *Carica*, and *Lychnis* (figs. 47-88) a Leitz compensating ocular no. 18 was used, the magnification obtained being about 4000 ×. In reproduction plates were reduced one-third.

PLATE IX

FIGS. 1-15, *Bryonia dioica*

- FIG. 1. Nucleus of pollen mother cell in early diakinesis.
- FIG. 2. Later diakinesis, showing budding of nucleolus.
- FIG. 3. Multipolar spindle; close association of paired chromosomes.
- FIG. 4. Cell with spindle figure showing relation of spindle diameter to cell diameter; chromosomes in late metaphase.
- FIGS. 5, 6. Chromosomes in metaphase, one chromosome in each group showing precocious splitting.
- FIG. 7. Chromosomes of figure 6 drawn separately.
- FIG. 8. Chromosomes in metaphase, one chromosome displaced in cutting.
- FIG. 9. Chromosomes of figure 8 drawn separately.
- FIG. 10. Chromosomes in late metaphase.
- FIG. 11. Chromosomes in metaphase from lightly stained preparation; split evident in 5 pairs of chromosomes, of which one is the precocious pair.
- FIG. 12. Chromosomes in metaphase from same preparation as figure 11.
- FIG. 13. Chromosomes in anaphase; longitudinal split evident.
- FIGS. 14, 15. Polar views of anaphase, each showing group of chromosomes passing to one pole. Cross sections of spindle fibers indicated in figure 14.

FIGS. 16-30, *Clematis virginiana*

- FIG. 16. Nucleus of pollen mother cell in diakinesis.
- FIG. 17. Chromosome pairs from various nuclei in diakinesis.
- FIG. 18. Cell with spindle figure showing relative size of spindle and cell; chromosomes in metaphase.
- FIGS. 19-22. Chromosomes in metaphase; 8 pairs shown in figure 19.
- FIG. 23. Individual chromosome pairs from different cells; *a*, *b*, and *c* in late metaphase, *d* and *e* in anaphase.
- FIG. 24. Chromosomes of figure 20 drawn separately.
- FIG. 25. Chromosomes from one cell, showing 6 atelomitic and 2 telomitic pairs.
- FIG. 26. Polar view of metaphase.
- FIG. 27. Late anaphase, longitudinal split evident.
- 12

- FIG. 28. Two adjoining cells showing polar and side views of late anaphase.
 FIGS. 29, 30. Polar views of late anaphase.

PLATE X

FIGS. 31-46, *Smilax herbacea*

- FIG. 31. Nucleus of pollen mother cell in diakinesis.
 FIG. 32. Chromosome pairs from different nuclei in diakinesis.
 FIGS. 33, 34. Chromosomes in metaphase.
 FIG. 35. Cell with spindle figure; chromosomes in late metaphase.
 FIG. 36. Chromosome pairs from different cells giving impression of inequality due to folding over of one member.
 FIG. 37. Chromosomes in late anaphase.
 FIGS. 38, 39. Polar views of metaphase.
 FIGS. 40, 41. Late anaphases.
 FIG. 42. Polar view of very late anaphase.
 FIG. 43. Chromosomes in homoeotypic metaphase.
 FIG. 44. Homoeotypic anaphase, polar and side view of chromosomes.
 FIG. 45. Polar view of one chromosome group in homoeotypic anaphase.
 FIG. 46. Homoeotypic anaphase, polar view of chromosome group in each cell.

FIGS. 47-55, *Menispermum canadense*

- FIGS. 47, 48, 50-52. Nuclei of pollen mother cells in diakinesis.
 FIG. 49. Two chromosome pairs from a nucleus in diakinesis, each showing lobing of one member.
 FIGS. 53, 54. Paired chromosomes free in cytoplasm following disappearance of nuclear membrane. Period of close approximation of members of pairs.
 FIG. 55. Cell with spindle figure; chromosomes in metaphase.

PLATE XI

FIGS. 56-71, *Menispermum canadense*

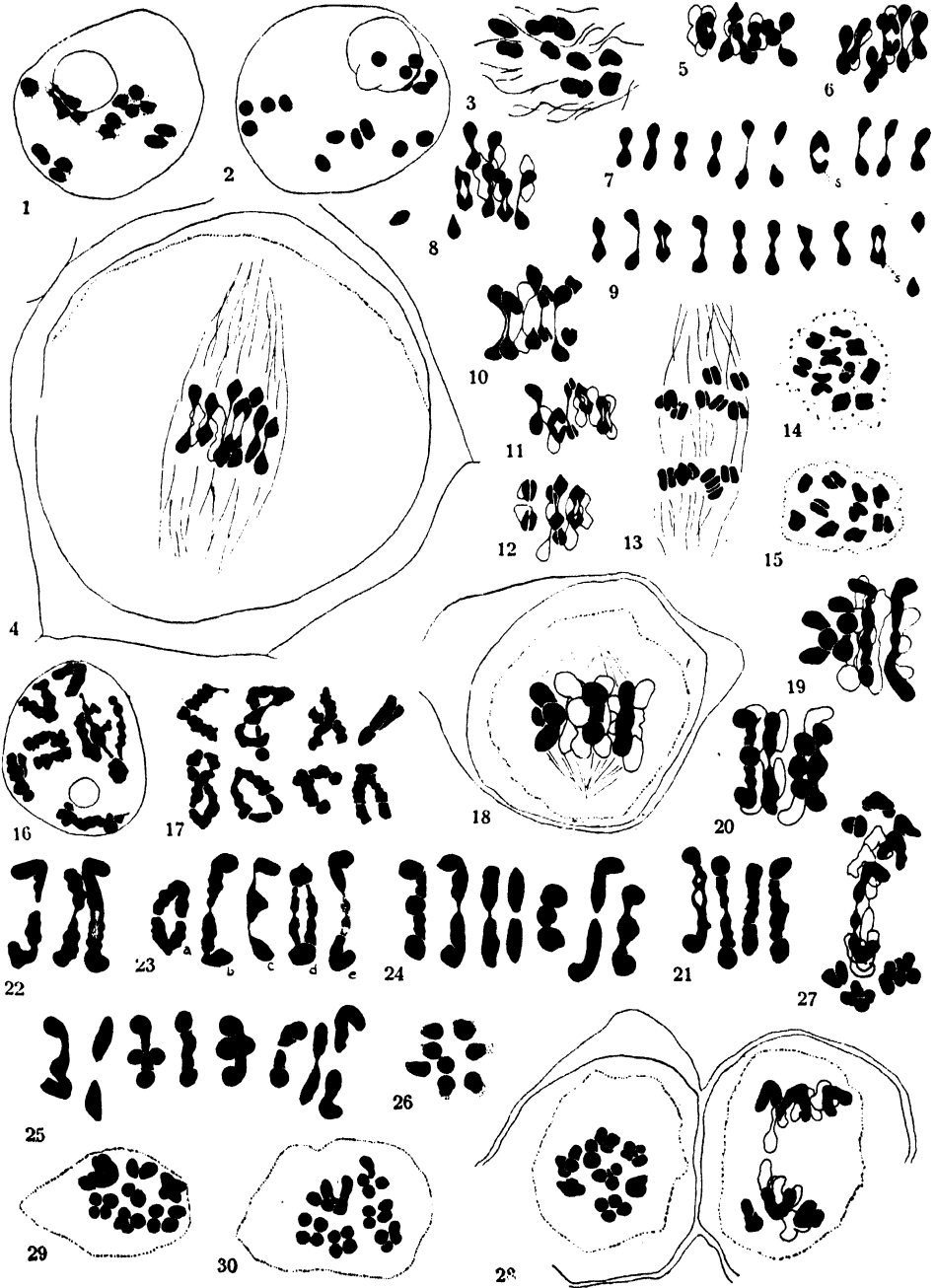
- FIGS. 56-61. Metaphase groups from different cells, showing varied appearance of peculiar chromosome pair.
 FIGS. 62-66. Chromosome groups in late metaphase or early anaphase. Figures 62 *a* and *b*, 64 *a* and *b*, 65 *a* and *b*, in each case from two successive sections of a spindle.
 FIGS. 67-69. Polar views of chromosome groups in metaphase.
 FIG. 70. Homoeotypic division, polar and side views of the chromosomes in metaphase.
 FIG. 71. Homoeotypic metaphase, polar views of both chromosome groups.

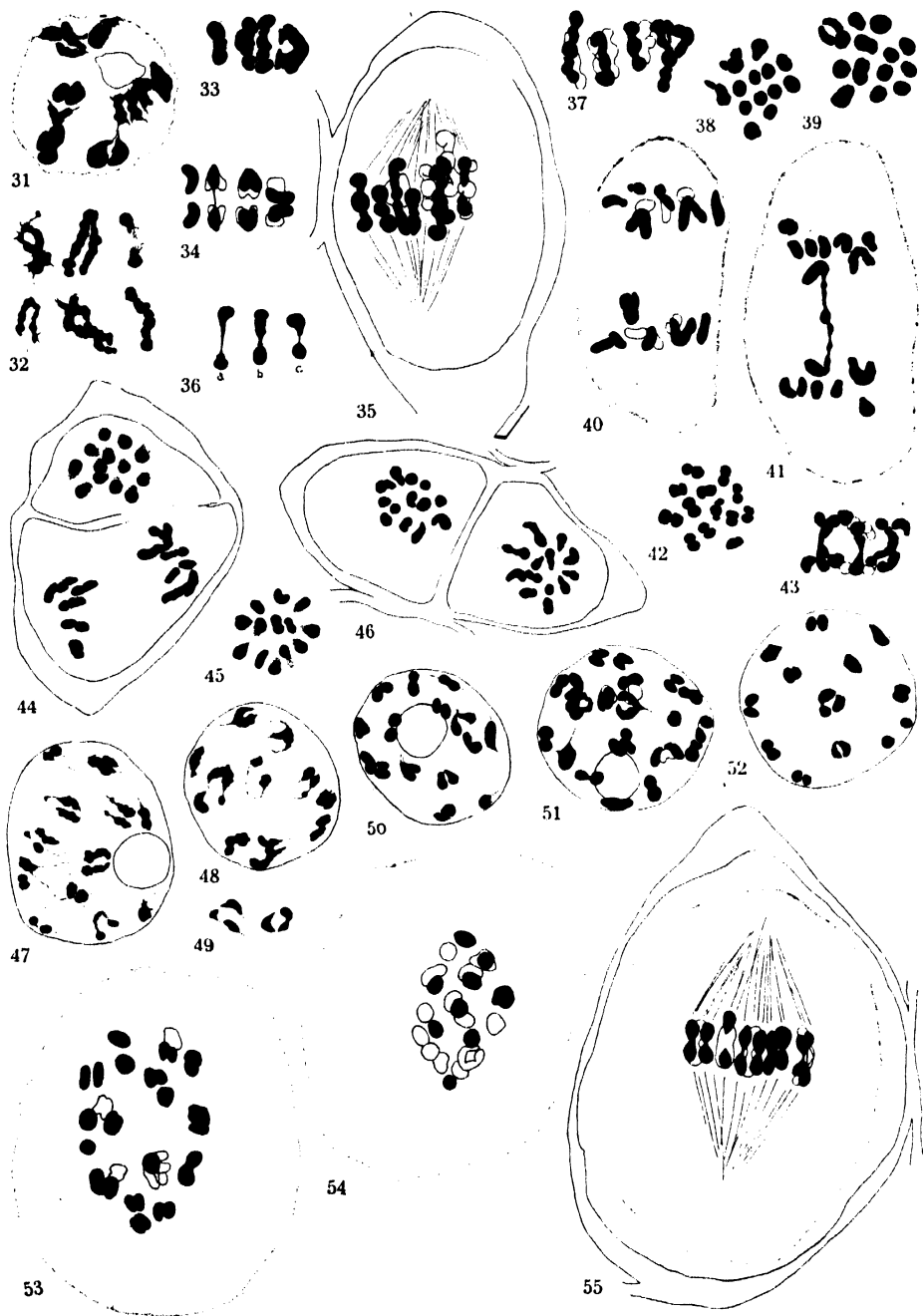
FIGS. 72-83, *Carica papaya*

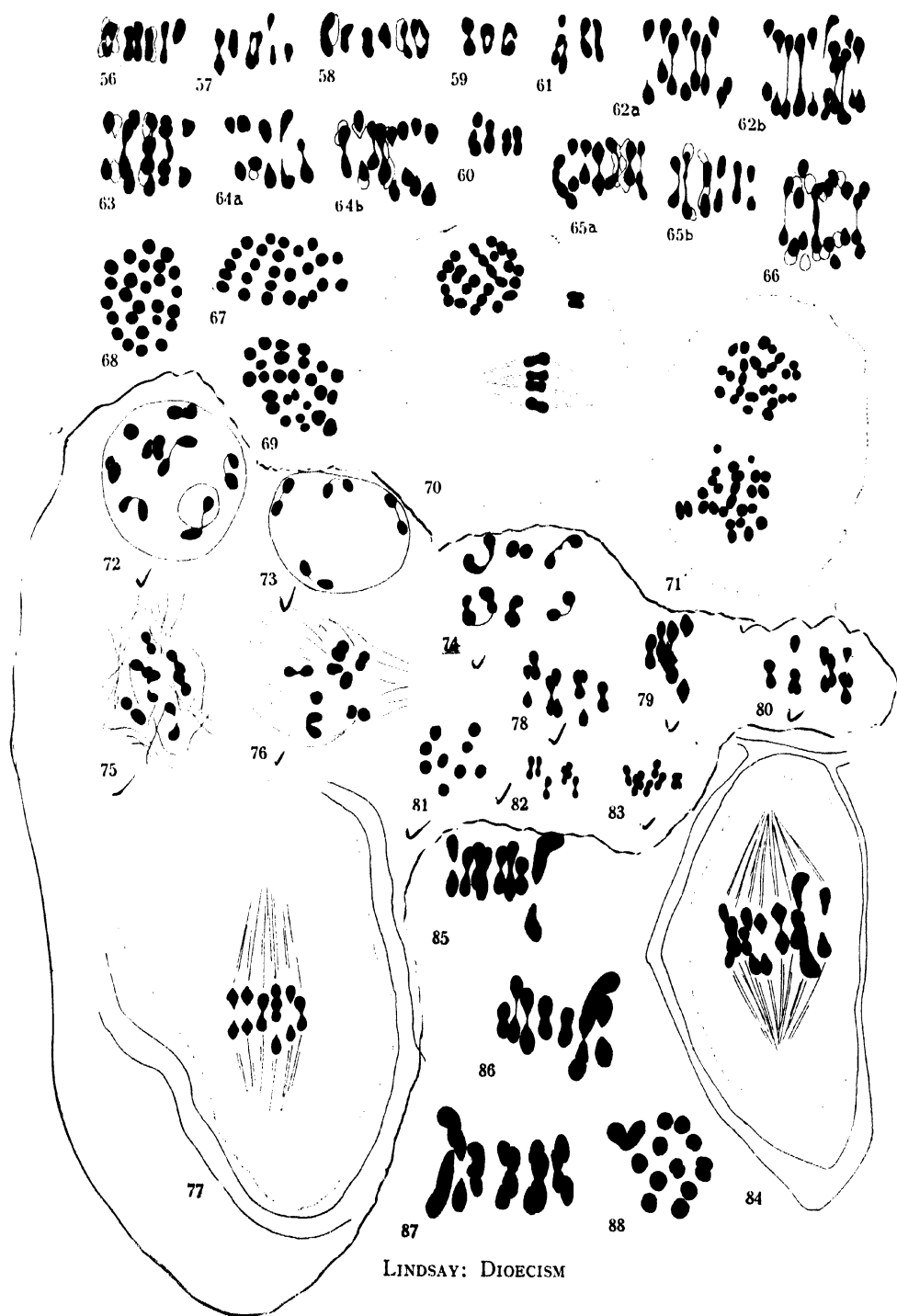
- FIGS. 72, 73. Nuclei of pollen mother cells in diakinesis.
 FIG. 74. Chromosome pairs from different nuclei in diakinesis.
 FIGS. 75, 76. Multipolar spindles; close association of chromosome pairs.
 FIG. 77. Cell with spindle figure; chromosomes in late metaphase or early anaphase.
 FIGS. 78-80. Chromosome groups in metaphase or early anaphase.
 FIG. 81. Polar view of metaphase group.
 FIGS. 82, 83. Homoeotypic metaphases.

FIGS. 84-88, *Lychnis alba*

- FIG. 84. Cell with spindle figure; chromosomes in metaphase or early anaphase.
 FIGS. 85-87. Chromosome groups in metaphase showing unequal pair.
 FIG. 88. Polar view showing anaphase group containing the larger member of the unequal pair.







LINDSAY: DIOECISM

THE BIOLOGICAL SIGNIFICANCE OF CERTAIN DIFFERENCES BETWEEN THE VALUES OF THE CORRELATION COEFFICIENT, CORRELATION RATIO, AND CONTINGENCY COEFFICIENT

J. ARTHUR HARRIS, CHI TU, AND MARIAN WILDER

(Received for publication September 23, 1929)

INTRODUCTION

Because of the great complexity of biological, social, and economic phenomena, and because of the various (and often illogical) ways in which measurements are made and data recorded by workers who are not trained in mathematical reasoning, it has been necessary to deduce a large number of biometric formulae in an effort to secure methods for dealing with all kinds of frequency distributions and frequency surfaces.

In comprehensive investigations it often becomes necessary to consider in a comparative way the values of constants determined by different methods, or based on data of different nature or origin. An essential to the drawing of sound biological conclusions from such comparisons is a full understanding of the limitations of applicability of the various formulae. Since all of the biometric equations in their theoretical development rest on antecedent assumptions, these must be borne in mind in the interpretation of the constants in terms of which the results of specific investigations are expressed. This is especially important when various constants (for example correlation coefficients, correlation ratios, and contingency coefficients) based on different kinds of data are compared.

The purpose of the present paper is to discuss the biological significance of the differences between the measures of interrelationship afforded by the correlation coefficient, correlation ratio, and contingency coefficient when applied to certain botanical data, and to indicate the reasons for the differences in the magnitudes of these coefficients, with a view to emphasizing the importance of morphological considerations in the selection of the specific method to be applied in any particular case.

RESULTS

Given a plant ovary of three locules, statistical data concerning its fertility may be recorded in terms of number of ovules formed per locule or per fruit, or in terms of the number of ovules developing to the stage of mature seeds. These numerical data, as such, do not, however, furnish a complete description. For any given locule the number of ovules formed may be either "odd" or "even." Since in the great majority of cases the

two carpellary margins will have the same number of ovules, or differ by only one ovule, the "odd" or "even" character merely indicates the bilateral symmetry or asymmetry of the carpel as expressed in terms of number of ovules produced on the two carpellary margins.

In the case of the unilocular fruit, evidence has been adduced to show that the odd or even character of the number of ovules found may have a physiological significance in the development of the ovules into seeds.^{1, 2, 3} In certain phases these investigations involved the determination of partial correlation coefficients from correlation coefficients derived by the bi-serial method of Pearson.⁴

At the time, this was done without theoretical justification. A subsequent empirical investigation by Newbold⁵ indicates the validity of the procedure.

These results, as well as others to be cited later, justify the investigation of the "locular composition" of the ovary. By locular composition we understand merely the numbers of "even" and "odd" locules of which the ovary is made up. Thus for a trimerous fruit the locular composition is $3e$, $2e + o$, $e + 2o$, or $3o$.

In the case of the multilocular fruit we further require some measure of the inequalities in the distribution of number of ovules per locule in the same ovary. This has been defined⁶ by a coefficient of radial asymmetry. By radial asymmetry we understand the root-mean-square deviation of the number of ovules per locule from the mean number of ovules per locule in the ovary under consideration.

In an investigation of the ovary of *Staphylea*^{6, 7, 8} it has been shown that there is a differential mortality with respect to both locular composition and radial asymmetry. In the course of any such investigation it becomes necessary to consider the correlation between the various characteristics

¹ Harris, J. A. On the relationship between bilateral asymmetry and fertility and fecundity. Arch. Entwicklungsmech. Organ. 35: 500-522. 1912.

² —. Further studies on the relationship between bilateral asymmetry and fertility and fecundity in the unilocular fruit. Genetics 2: 186-204. 1917.

³ —. On the applicability of Pearson's biserial r to the problem of asymmetry and fertility in the unilocular fruit. Genetics 2: 205-212. 1917.

⁴ Pearson, K. On a novel method of determining correlation between a measured character A, and a character B, of which only the percentages of cases wherein B exceeds (or falls short of) a given intensity is recorded for each grade of A. Biometrika 7: 96-105. 1909.

⁵ Newbold, E. M. Notes on an experimental test of errors in partial correlation coefficients derived from four-fold and biserial tables. Biometrika 17: 251-265. 1925.

⁶ Harris, J. A. On the selective elimination occurring during the development of the fruit of *Staphylea*. Biometrika 7: 452-504. 1910.

⁷ —. The selective elimination of organs. Science 32: 519-528. 1910.

⁸ —. Further observations on the selective elimination of ovaries in *Staphylea*. Zeitschr. Ind. Abst.-Vererb. 5: 173-188. 1911.

TABLE 1. *Relationship Between Radial Asymmetry and Locular Composition in Staphylea (Series B)*

Radial Asymmetry

| | .0000 | .4714 | .8165 | .9428 | 1.2472 | 1.4142 | 1.6330 | 1.6997 | 1.8856 | 2.0548 | 2.1602 | Total |
|---------------------|-------|-------|-------|-------|--------|--------|--------|--------|--------|--------|--------|-------|
| Locular Composition | | | | | | | | | | | | |
| 3 even, 0 odd..... | 610 | — | — | 130 | — | — | — | — | 1 | — | — | 741 |
| 2 even, 1 odd..... | — | 739 | 146 | — | 35 | 9 | — | 6 | — | 1 | — | 936 |
| 1 even, 2 odd..... | — | 451 | 142 | — | 28 | 7 | — | 2 | — | 2 | 1 | 633 |
| 0 even, 3 odd..... | 110 | — | — | 42 | — | — | 3 | — | — | — | — | 155 |
| Total..... | 720 | 1190 | 288 | 172 | 63 | 16 | 3 | 8 | 1 | 3 | 1 | 2465 |

of the ovary for which a differential mortality is demonstrated, for the purpose of determining whether the selective death rate observed for the several characters dealt with is actually referable to the given character in each case or whether it is merely apparent because the given character is correlated with some other character for which the death rate is directly selective. The method of determining the correlation must obviously receive consideration.

In the dissection of 7264 trimerous ovaries, the classes of locular composition and radial asymmetry, shown in the accompanying table 1 for Series B, and in two other tables (XLI and XLIII, for Series A and C) published in the original memoir, were found.

Table 2 gives the correlation coefficients, correlation ratios, and contingency coefficients measuring the relationship between locular composition, c , and radial asymmetry, a , in the three series of ovaries. Here r_{ac} is the

TABLE 2. *Comparison of Correlation Coefficients, Correlation Ratios and Contingency Coefficients Measuring the Relationship Between Radial Asymmetry and Locular Composition*

| Series | N | Correlation Coefficient r_{ca} | Correlation Ratio $c\eta_a$ | Correlation Ratio $a\eta_c$ | Contingency Coefficient C_1 |
|---------------------|------|--|-----------------------------------|-----------------------------------|-------------------------------------|
| Eliminated (A)..... | 2095 | .2455 | .4906 | .4541 | .7082 |
| Developing (B)..... | 2675 | .3295 | .5447 | .4720 | .7133 |
| Matured (C)..... | 2704 | .3280 | .5112 | .6603 | .7073 |

product moment correlation coefficient, $c\eta_a$ is the correlation ratio⁹ determined from the means of the arrays of radial asymmetries associated with various classes of locular composition, $a\eta_c$ is the correlation ratio determined from the means of locular composition associated with various classes of asymmetry, and C_1 is the first coefficient of contingency.¹⁰

The three constants, r , η and C_1 , show large discrepancies in their magnitudes. The correlation ratios are about twice as large as the correlation coefficients. The contingency coefficients are conspicuously larger than either of the other constants. They are about fifty percent higher than the correlation ratios, and are nearly three times as large as the correlation coefficients. The probable errors of the coefficients have not been given since they would serve no useful purpose in the present connection unless the correlations of the three coefficients among themselves were known.

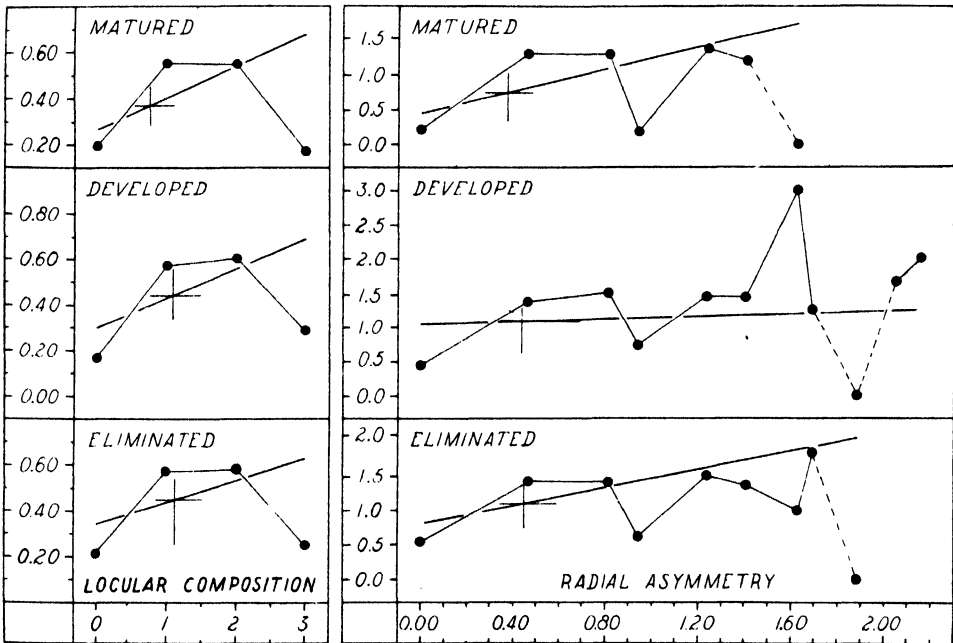
A glance at table 1 shows that certain combinations of radial asymmetry and locular composition do not occur. The reason is a purely physical

⁹ Pearson, K. On the general theory of skew correlation and non-linear regression. Drapers' Co., Res. Mem., Biom. Ser. 2. London, 1905.

¹⁰ Pearson, K. On the theory of contingency and its relation to association and normal correlation. Drapers' Co., Res. Mem., Biom. Ser. 1: 1-35. PIs. 1-2. 1904.

or numerical one. Ovaries which are made up of two locules with an even number of ovules and one locule with an odd number of ovules, or of one locule with an even and two locules with an odd number of ovules, cannot have a coefficient of asymmetry of 0.0000, since one of the locules must differ from the other two by at least one ovule. Similarly, trimerous ovaries which are made up either of three locules with even numbers of ovules or of three locules with odd numbers of ovules may have coefficients of radial asymmetry of 0.0000, 0.9428, 1.6330, or 1.8856, but cannot have coefficients of asymmetry of 0.4714, 0.8165, 1.2472, 1.4142, 1.6997, 2.0548, or 2.1602, since to maintain the specified locular composition one of the locules must differ from the other two in number of ovules by at least two ovules. Other details will be clear from the original paper.

Now these physical limitations, imposed by the fact that number of ovules is necessarily recorded in discontinuous classes, must influence



TEXT FIG. 1. Regression of radial asymmetry on locular composition (left) and of locular composition on radial asymmetry (right), for eliminated, developing, and matured ovaries of *Staphylea*.

profoundly the magnitude of the coefficients measuring the relationship between the two variables.

A discrepancy between the coefficient of correlation and the correlation ratios is to be expected if the relationship between the two variables is not linear.

That the differences in the present values of the correlation coefficients and correlation ratios are due to the nature of the regression is suggested

by the regression straight lines represented graphically in text figure 1 for the regression of radial asymmetry on locular composition (left side) and for the regression of locular composition (in terms of number of "odd" locules per ovary) on radial asymmetry (right side). Both indicate large deviations of the empirical means from the regression lines.

In the regression of radial asymmetry (scale of ordinates of left figure of diagram) on locular composition, the mean asymmetries are uniformly high for ovaries with 1 and 2 odd locules and uniformly low for ovaries with 0 and 3 odd locules. In the regression of locular composition (scale of ordinates of right figure of diagram) on radial asymmetry the three series show greater irregularities but all show large deviations from the regression line.

Blakeman's test for linearity of regression ¹¹ gives the following results.

| | For $\epsilon\eta_a$ | For $\alpha\eta_e$ |
|----------------|----------------------|--------------------|
| Series A | 17.31 | 14.46 |
| Series B | 19.08 | 13.82 |
| Series C | 16.08 | 27.53 |

These ratios can leave no question concerning the significantly non-linear nature of the relationship between locular composition and radial asymmetry. Non-linearity of regression, indeed, is morphologically necessary.

Remembering that for the contingency surface of p classes of x and q classes of y

$$\chi^2 = \sum_1^p \left\{ \sum_1^q \left[\frac{(n_{nv} - n_{nv}')^2}{n_{nv}'} \right] \right\},$$

where n_{nv} is the actual frequency of individuals having the characteristics of the n th class of x and the v th class of y , and n_{nv}' is the frequency calculated on the assumption of complete independence of x and y , it is clear that the contribution of cells which are void for any reason will be

$$\sum_1^p \left[\sum_1^q (n_{nv}') \right]$$

and that this may be a relatively large part of the value of χ^2 on which the magnitude of

$$C_1 = \left(\frac{\chi^2/N}{1 + \chi^2/N} \right)^{1/2}$$

depends.

There can be no doubt that the large value of the contingency coefficient as compared with the other measures of interrelationship is due to the contribution of the cells which for physical reasons cannot contain frequencies to the value of χ^2 . The percentage contributions of these classes are:

¹¹ Blakeman, J. On tests for linearity of regression in frequency distributions. *Biometrika* 4: 332-350. 1905.

| | Total χ^2 | Contribution of void cells | Percentage contribution of void cells |
|-----------------------|-------------------|-------------------------------|--|
| Series <i>A</i> | 2108.36 | 953.74 | 45.24 |
| Series <i>B</i> | 2552.99 | 1141.97 | 44.73 |
| Series <i>C</i> | 2706.79 | 1352.01 | 49.95 |

Thus about half of the total value of χ^2 is contributed by the void cells.

It is clear that as measures of the interdependence of x and y , the values of r_{ac} , $c\eta_a$, $a\eta_c$, and C_1 are largely determined by factors which are rigidly fixed by the conditions inseparable from the numerical morphological characteristics of the organ on which the coefficient is based.

As far as we are aware there is as yet no mathematical theory to permit the selection of the most probably correct method of removing the theoretical frequencies from the cells in which actual frequencies cannot occur and redistributing them in the cells in which they may occur. Empirically we note that we may remove the numerical-morphological limitation to the applicability of the contingency method by splitting our materials up into classes in such a way that this limitation will not obtain.

First, we may condense the theoretical frequencies of void cells into the cells in which these may actually occur.

In the absence of any certainty as to which of the indefinitely large number of ways of distributing the theoretical frequencies of the void cells among the occupied cells is the best method, some one procedure which has at least the merit of being unique and constant in its application must be tested. Such a method is that of concentrating the theoretical frequencies from the void cells into the theoretically occupied cells in proportion to the numbers theoretically already present. Thus the corrected theoretical frequency for any occupied cell will become

$$n_{nv}'' = n_{nv}' \frac{N}{S_o(n_{nv}')}$$

where n_{nv}'' denotes the theoretical number in the occupied cells after correction and S_o denotes summation for theoretically occupied cells. Whatever may be the objections to this method, it meets the necessary requirement $S(n_{nv}'') = N$.

Second, we may attempt to remove the numerical-morphological limitation to the applicability of the contingency method by splitting our materials up into classes such that the specified limitation will not obtain.

By separating the classes of locular composition into two groups

Group *A*: 3 odd, 3 even

Group *B*: 2 odd + 1 even, 1 odd + 2 even

we may divide each contingency surface into two bi-serial tables, *A* and *B*, each of which will have a smaller number of asymmetry classes but each

of which will represent distributions which may have frequencies in every cell.¹² Thus the (4 x 11)-fold surface given in table 1 may be broken into the (2 x 4)-fold and the (2 x 7)-fold distributions shown as tables 3 and 4.

TABLE 3. *Bi-serial Contingency Surface from Table 1*
Radial Asymmetry

| | .0000 | .9428 | 1.6330 | 1.8856 | Total |
|---------------------|-------|-------|--------|--------|-------|
| Locular Composition | | | | | |
| 3 even, 0 odd | 610 | 130 | — | 1 | 741 |
| 0 even, 3 odd | 110 | 42 | 3 | — | 155 |
| Total | 720 | 172 | 3 | 1 | 896 |

TABLE 4. *Bi-serial Contingency Surface from Table 1*
Radial Asymmetry

| | .4714 | .8165 | 1.2472 | 1.4142 | 1.6997 | 2.0548 | 2.1602 | Total |
|---------------------|-------|-------|--------|--------|--------|--------|--------|-------|
| Locular Composition | | | | | | | | |
| 2 even, 1 odd | 739 | 146 | 35 | 9 | 6 | 1 | — | 936 |
| 1 even, 2 odd | 451 | 142 | 28 | 7 | 2 | 2 | 1 | 633 |
| Total | 1190 | 288 | 63 | 16 | 8 | 3 | 1 | 1569 |

The relationship between the two variables (locular composition and radial asymmetry) involved in such tables might be treated by Pearson's method of determining the bi-serial r ¹³ or bi-serial η ¹⁴ with due regard to any limitations on the applicability of these methods inherent in our present data.

Using the marginal frequencies and N_a , N_b for the two bi-serial tables, we recalculate two values of χ^2 . Since the values of C_1 may not properly be determined by the usual theory for a $2 \times n$ -fold table, we may base our first evaluation on the relationship between a and c for the two separate bi-serial distributions on P ,¹⁵ as is wholly legitimate for such cases.

The results appear in table 5. Here the value of P is determined from Elderton's table¹⁶ with n' equal to the number of cells of the bi-serial

¹² For this suggestion we are indebted to Mr. Alan E. Treloar.

¹³ **Pearson, K.** On a novel method of determining correlations between a measured character A, and a character B, of which only the percentage of cases wherein B exceeds (or falls short of) a given intensity is recorded for each grade of A. *Biometrika* 7: 96-105. 1910.

¹⁴ ——. On a new method of determining correlation when one variable is given by alternative and the other by multiple categories. *Biometrika* 7: 247-257. 1910.

¹⁵ **Pearson, K.** On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. *Phil. Mag.* 50: 157-175. 1900.

¹⁶ **Elderton, W. P.** Tables for testing the goodness of fit of theory to observation. *Biometrika* 1: 155-163. 1901. Reprinted as table XII in Pearson's "Tables for Statisticians and Biometricians."

distribution. This procedure is not in accord with R. A. Fisher's conclusion¹⁷ that for such $(r \times s)$ -fold tables $n' = (r - 1)(s - 1) + 1$.

But our real need is not fully met by the information provided by

TABLE 5. *Values of χ^2 , P , and C_1 Derived from Bi-serial Contingency Surfaces*

| Series | Classes | N | χ^2 | P | C_1 |
|---------------------------|---|------|----------|-----------------------|-------|
| Eliminated <i>A</i> . . . | 3 <i>o</i> , 3 <i>e</i> | 734 | 1.3791 | .980564 | .0433 |
| | <i>o</i> + 2 <i>e</i> , 2 <i>o</i> + <i>e</i> | 1361 | 6.1140 | .728201 | .0669 |
| | Total surface | 2095 | 7.4905 | .975209 | .0597 |
| Developing <i>B</i> . . . | 3 <i>o</i> , 3 <i>e</i> | 896 | 22.6940 | .001961 | .1572 |
| | <i>o</i> + 2 <i>e</i> , 2 <i>o</i> + <i>e</i> | 1569 | 16.2103 | .238653 | .1011 |
| | Total surface | 2465 | 39.3604 | .010559 | .1254 |
| Matured <i>C</i> | 3 <i>o</i> , 3 <i>e</i> | 1358 | .4111 | .984612 | .0549 |
| | <i>o</i> + 2 <i>e</i> , 2 <i>o</i> + <i>e</i> | 1346 | .9805 | very large .994930 | .0850 |
| | Total surface | 2704 | 1.3902 | very large .999920 | .0715 |

these bi-serial tables as such. We require some measure of contingency for the surface as a whole which will be free from the influence of the contribution of the void cells. Let α denote the biserial table of N_α entities for cases in which all the cells of the ovary have the same locular composition and let β designate the biserial table of N_β entities in which the cells are not of the same locular composition.

Now in these two bi-serial distributions, α and β , into which the surface has been broken, the theoretical frequencies have been properly distributed in the cells in which they may occur. For each of the two bi-serial distributions $S_\alpha(p_{nv}) = 1$, $S_\beta(p_{nv}) = 1$.

If these probabilities were merely again combined by distributing the actual frequencies and the probabilities and calculated frequencies in tables of four classes of locular composition, $S_\alpha(p_{nv}) + S_\beta(p_{nv})$ would be 2. Furthermore no weight would be given to the difference between N_α and N_β . We therefore take

$$p_{\alpha}'' = \frac{N_\alpha}{N} p_{nv},$$

$$p_{\beta}'' = \frac{N_\beta}{N} p_{nv},$$

which fulfill the necessary requirements that $S(p'') = 1$.

This leaves the values of n_{nv}' for the whole surface identical with those calculated for the bi-serial tables, and for the surface as a whole

$$\chi^2 = \chi_\alpha^2 + \chi_\beta^2.$$

¹⁷ Fisher, R. A. On the interpretation of χ^2 from contingency tables and the calculation of P . Jour. Roy. Stat. Soc. 85: 87-94. 1922.

Comparing the results for those two methods of redistributing the theoretical frequencies, we find the values of the contingency coefficients shown in the following table.

TABLE 6. *Comparison of Corrected and Uncorrected Contingency Coefficients*

| Series | Original Contingency C_1 | Method B Theoretical Frequencies Computed from Separate Bi-serial Tables i.e. from $p_{\alpha'}', p_{\beta'}'$ C_1 | Method A Probabilities Computed for Filled Cells by Taking $p_{nv}' = p_{nv} \frac{1}{S_0(p_{nv})}$ C_1 |
|------------------------|----------------------------------|---|---|
| Eliminated A | .7082 | .0597 | .0931 |
| Developing B | .7133 | .1254 | .2864 |
| Matured C | .7073 | .0715 | .0231 |

The elimination of the influence of the void cells by these two, wholly empirical, methods has reduced the contingency coefficients to very low magnitudes.

DISCUSSION

We now have to consider the biological interpretation of these statistical results.

First of all it is to be noted that the three statistical procedures here applied to the measurement of the interrelationship between locular composition and radial asymmetry in the fruit of *Staphylea* lead to very different numerical values. The orders of magnitude of the constants are so diverse, and the results for any one method so consistent from series to series, that it is not necessary to compare the results due to the three methods with regard to their probable errors.

The contingency coefficient, C_1 , is roughly fifty percent higher than the correlation ratio, $c\eta_a$, $a\eta_c$, and about two or three times as large as the correlation coefficient, r_{ac} . This high value of the contingency coefficient is primarily attributable to the high percentage contribution of void cells to the value of χ^2 . The present case represents, therefore, a second class of limitations in the applicability of the contingency method as at present developed and applied. No correction for the influence of these void cells is obvious.

From these results, as well as from those of a preceding paper,¹⁸ it is evident that in the use of the contingency coefficient in biological or other investigations, care should be used to ascertain whether any of the cells of the surface are necessarily void.

Nevertheless, these results should not be used as a basis for the condemnation of the contingency method. Much depends upon the purpose for which it is to be applied. If the thing which is required in the investigation be a measure of the deviation of the given system from independent

¹⁸ Harris, J. A., and A. E. Treloar. On a limitation in the applicability of the contingency coefficient. Jour. Amer. Stat. Assoc. 22: 460-472. 1927.

probability, irrespective of the origin of this deviation, the contingency coefficient possibly furnishes the best measure of the three.

The difference between the correlation coefficient r_{ac} , and the ratios, ${}_c\eta_a$, ${}_a\eta_c$, are clearly attributable to the conspicuously non-linear nature of the regression of these two characters. These departures from linearity obviously rest on a morphological foundation.

Probably the result of greatest significance of the present study is the evidence which it affords of the importance of a careful consideration of the morphological nature of the data to which statistical methods of analyses are to be applied.

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GUM, TANNIN, AND RESIN IN RELATION TO SPECIFICITY, ENVIRONMENT, AND FUNCTION

JAMES B. MCNAIR

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The object of this paper is to show the taxonomic and climatic distribution of plants containing relatively large amounts of gum, tannin, and resin, and to determine the bearing of such facts on the functional importance of these substances.

The chemical composition of gum, tannin, and resin leads one to consider a possible mutual association between them in the plant, for gums may be compounds of sugars, as is the case of pentosans; tannins may have sugar combined in their molecules, as is the case of tannin glucosides; and resins may contain tannin as well as gum in their aggregates, as is probably the case of gambir, gamboge, galbanum, and kino. It was thought worth while to investigate the occurrence of gum, tannin, and resin in plant families, genera, and species to determine a possible taxonomic relation between these constituents.

A study of the distribution of these substances in plants is also of interest in connection with their possible function. Gum is said to serve as a reserve food or to check excessive transpiration; tannin to protect against frost, animals, and fungi; and resin as protection in case of injury, and against animals and excessive transpiration. Protection against excessive transpiration is evidently most advantageous in desert plants and in evergreen plants in cold climates, so that agents useful in checking transpiration should be more common in these plants. Gum and resin should therefore be more abundant in plants subjected to excessive transpiration. Likewise a substance functioning as a protection against frost, as tannin is supposed to do, should be more abundant in temperate than in tropical regions. For these ecological reasons, therefore, a consideration of the distribution of plants containing gum, tannin, and resin is of interest.

It is quite probable that most, if not nearly all, plants contain small amounts of gum, tannin, resin, and oil, and it is well known that a smaller number contain comparatively large amounts of these substances. It is widely known that some plants may have large amounts of one of these

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materials and not of the others, but a general correlation with other traits has not heretofore been made. In attempting such a correlation the writer has assumed that the list of plant oils compiled by Lewkowitch is fairly complete; that the data on plants containing gum in appreciable quantities collected by Brehmer and Zeisel is representative; that the catalogue of tannin-bearing plants assembled by Brehmer and Konstanty lacks no important members; and that the statistics of resinous plants gathered together by Wolff are sufficient.

GUM

Chemical Definition

Four distinct classes of gums are recognized: (1) gums containing arabin or arabic acid, (2) gums consisting of mixtures of arabin and cerasin (cerasic acid), (3) gums containing bassorin, and (4) gums containing mixtures of cerasin and bassorin.

Gums of the first class include gum arabic from *Acacia* (Leguminosae), feronia gum from *Ferronia elephantum* (Rutaceae), and anacardium gum from *Anacardium occidentale* (Anacardiaceae). It may be of interest to know that *Acacia* has also tannin and resin, the Rutaceae have tannin but no resin, and the Anacardiaceae have both tannin and resin.

Gums of the second class include those from the rose family such as the cherry, almond, apricot, and plum. Some of these have tannin but none has resin.

Gums of the third class include gum tragacanth from *Astragalus* spp. (Leguminosae), cocopalme gum from *Cocos nucifera* (Palmae), chagual gum from *Puya coarctata* (Bromeliaceae), and moringa gum from *Moringa pterygosperma* (Moringaceae). Of these plants none has tannin or resin.

Gums of the fourth class include East India gum from *Cochlospermum Gossypium* (Cochlospermaceae). These plants contain no tannin or resin.

To summarize: Plants containing the first class of gums may contain no tannin or resin, tannin but no resin, or both tannin and resin. Plants of the second class do not have resin but may or may not have tannin. Plants of the third class have neither tannin nor resin. Plants of the fourth class have no tannin or resin.

Families and Genera

Of approximately 280 plant families listed by Engler and Prantl, 19, or 6.8 percent, contain appreciable quantities of gum.

Of these 19 gum-containing families, 5, or 26 percent, contain resin and 11, or 58 percent, have tannin.

Of the 338 genera examined, 57, or 17 percent, contain gum and of these 10, or 18 percent, contain tannin and 2, or 3.5 percent, contain resin. These percentages certainly indicate that it seems by no means necessary for a plant to contain appreciable quantities of either tannin or resin if it contains gum.

Only 4 families that contain gum also contain tannin and resin; and when the number of gum genera that contain both tannin and resin are investigated, only one is known, namely, *Acacia*.

From the standpoint of ecology it is of interest to note that of the gum-containing families 15, or 79 percent, are of tropical habitat and 1, or 5 percent, is found in the temperate zone. This fact is also borne out by statistics of the gum plants that contain oil, for only 16 percent have drying oils. It has been shown in a previous paper by the writer (1929) that tropical plant families contain by far the greatest percentage of non-drying oils or fats.

TANNIN

Chemical Definition

Tannins are water-soluble principles that occur in the cell-sap, especially of parenchyma cells, of a large number of plants. They are derivatives of phenol and phenol acids, and give either dark blue or green precipitates with solutions of ferric chlorid. They were formerly distinguished according to the plants from which they were obtained; thus we recognized chestnut tannin, oak tannin, etc. Recent studies on the constitution of these substances show that there are two principal groups of tannins, (1) those in the nature of glucosides and (2) others not yielding any dextrose on hydrolysis with acids. Both of these groups may be subdivided into two classes, namely, (a) those which probably are derivatives of protocatechuic acid and (b) those which are derivatives of gallic acid.

Families and Genera

Of approximately 280 plant families listed by Engler and Prantl, 80, or 29 percent, contain appreciable quantities of tannin. Of the 338 genera examined, 229, or 68 percent, contain tannin. But few of these tannin-containing families have gum or resin in appreciable quantities. The approximate figures are 11, or 14 percent, for gum, and 16, or 20 percent, for resin. Of the 229 tannin genera, 10, or 4 percent, contain gum, and 14, or 6 percent, contain resin. This certainly indicates that it is by no means necessary for a plant to contain appreciable quantities of either gum or resin if it contains tannin.

Although a considerable number of tannin families contain either gum or resin, the number of such families that have both gum and resin is decidedly small, namely 4, or 5 percent. When one investigates the number of tannin genera that contain both gum and resin, only one is known, namely *Acacia*.

From the standpoint of ecology it is of interest to note that of the tannin-containing plants, 47, or 59 percent, are of tropical habitat and 20, or 25 percent, are found principally in the temperate zone. This fact is also borne out by statistics of the tannin plants that contain oil, as 57 percent have non-drying oils or fats and only 19 percent have drying oils. It has

been shown in a previous paper by the writer that tropical plant families contain by far the greater percentage of non-drying oils or fats.

Location

Considerable quantities of tannin are present in epidermal cells, especially in leaves, in dermal glands, in cork cells, in latex, in sacs near the vascular strands, in hypertrophies of medullary rays, and in heartwood.

Function

Very often considerable quantities of tannin are present in epidermal cells, especially in the case of leaves which persist through the winter. Warming inclines to the opinion that compounds of this nature serve to diminish the risk of desiccation, a danger by which arctic and alpine plants in particular are often threatened, especially in the absence of snow. Stahl on the other hand regards tannin in this first instance as a means of defense against the attacks of snails; it might also quite conceivably assist in preventing parasitic fungi from gaining access to the epidermal cells. Tannin may also assist against the assaults of animals of every kind, because of its strongly astringent taste and irritating properties on the digestive tract. Some consider tannin in particular cases as a reserve material capable of further utilization, but in other cases as purely an excretion product.

From the fact that tannin is contained in large amounts in plants of temperate habitat, in the bark and in the epidermis of leaves which persist through the winter, it would appear that tannin may serve as a protection from frost. Since many tropical plants contain large quantities of tannin it would also seem to have some other function, as perhaps protection against animals and fungi. The low number of tannin-containing genera that also have either gum or resin in appreciable quantities indicates that it is not necessary for a plant to contain either tannin and gum, tannin and resin, or tannin, gum, and resin.

RESIN

Chemical Definition

Seven principal groups of resins have been recognized: (1) tannol resins, esters of aromatic phenols that behave toward iron salts and some other reagents like tannins; (2) resene resins; (3) resinolic acid resins; (4) resinol resins; (5) fatty resins; (6) pigment resins; and (7) glucosidal resins.

Resins of the first class include Peru and Tolu balsams from *Toluiifera* (Leguminosae), styrax (Hamamelidaceae), benzoin (Styracaceae), aloe (Liliaceae), dragon's blood (Palmae), and ammoniac, galbanum, asafoetida, etc. (Umbelliferae). It may be of interest to know that most of the resins of this class are found in plants that have neither tannin nor gum. The exceptions are the Hamamelidaceae that have tannin but no gum and the Leguminosae that have both tannin and gum.

Resins of the second class include myrrh, olibanum, and elemi (Burseraceae), mastic (Anacardiaceae), and gurjun balsam, dammar (Dipterocarpaceae). Besides resin, the Burseraceae have no tannin or gum, the Anacardiaceae have both, and the Dipterocarpaceae have tannin but no gum.

Resins of the third class include sandarac, Canada turpentine, and Strassburg turpentine from the Coniferae and copaiba from the Leguminosae. The Coniferae have tannin but no gum and the Leguminosae have both tannin and gum.

Resins of the fourth class include guaiac from *Guaiacum* of the Zygophyllaceae. This family has no tannin or gum.

Resins of the fifth class include stick lac or shellac. This resin is an insect excretion and is part plant and part animal in origin. The plants include *Butea* (Leguminosae), *Ficus* (Moraceae), and *Acacia* and *Cajanus* (Leguminosae).

Resins of the sixth class include gamboge from *Garcinia* (Guttiferae). The Guttiferae have tannin but no gum.

Resins of the seventh class include jalap, scammony, etc., of the Convolvulaceae and are not associated with plants that have gum or tannin.

To summarize: Most plants containing tannol resins have no tannin or gum, although they may possess both; resene resins may be found in plants having neither gum nor tannin, tannin but no gum, or both gum and tannin; resinolic acid resins have as their source plants those that have tannin but no gum or plants that have both tannin and gum; resinol resins come from plants that have neither tannin nor gum; fatty resins have as source plants several tropical plants which may contain both tannin and gum or only tannin; pigment resins are borne by plants which have tannin but no gum; and glucosidal resins are perhaps not found in appreciable quantity in plants that have gum or tannin.

Families and Genera

Of approximately 280 plant families listed by Engler and Prantl, 27, or 10 percent, contain appreciable quantities of resin. Of the 338 genera examined, 84, or 25 percent, contain resin.

A number of these resin-containing families have tannin or gum in appreciable quantities. The approximate figures are 5, or 18 percent, for gum and 16, or 60 percent, for tannin. Of the 84 resin-containing genera, 2, or 2 percent, contain gum and 14, or 17 percent, contain tannin. This certainly indicates that it is by no means necessary for a plant to contain considerable quantities of either tannin or gum if it contains resin.

Although an appreciable number of resin families contain either tannin or gum, the number of such families that have both tannin and gum is decidedly small, namely 4 or 5 percent. When one investigates the number of resin genera that contain both tannin and gum, only one is known, namely, *Acacia*.

TABLE I. *Relation between Taxonomic Position, Climate of Habitat, and Possession of Tannin, Gum, Resin, and Oil. Numbers Are of Species*

| Family | Tannin | Gum | Resin | Drying Oil | Semi-Drying Oil | Non-Drying Oil | Fat | Wax |
|-----------------|----------------------------------|---------------------------------|-------------------------------|------------|------------------|---------------------------------|-----|------------------------------|
| Cycadaceae | — | 1 | — | — | — | — | — | — |
| Taxaceae | 6† | — | — | 2* | — | — | — | — |
| Pinaceae | 8† | — | 37 | 2† | — | — | — | — |
| Gramineae | — | — | — | 1 | 6 | 3 | — | 2 |
| Cyperaceae§ | — | — | — | — | — | 1 | — | — |
| Palmae† | 5† | 3† | 4† | — | 1† | 2† | 13† | 4† |
| Bromeliaceae† | — | 4† | — | — | — | — | — | — |
| Liliaceae†* | — | — | 3† | 1 | — | — | — | — |
| Iridaceae§ | 2* | — | — | — | — | — | — | — |
| Zingiberaceae† | 5† | — | — | — | — | — | — | — |
| Casuarinaceae† | 4† | — | — | — | — | — | — | — |
| Salicaceae* | 25* | — | — | — | — | — | — | — |
| Myricaceae† | 5† | — | — | — | — | — | 6† | — |
| Juglandaceae* | 5†* | — | 1† | 2* | 2* | — | — | — |
| Betulaceae* | 11 | — | 1 | — | — | 2 | — | — |
| Fagaceae* | 37 | — | — | — | 1 | 1 | — | — |
| Ulmaceae§ | 7 ^{2†} _{5*} | — | — | — | — | 1* | — | — |
| Moraceae§ | 13 ^{12†} _{1*} | — | 1† | 3* | — | — | — | 2 [†] _{2*} |
| Olacaceae†† | — | — | — | 1† | — | 3† | 1† | — |
| Chenopodiaceae | — | — | — | — | — | — | 3† | — |
| Amarantaceae | — | — | — | 1† | — | — | — | — |
| Ranunculaceae* | — | — | — | — | 2† | — | — | — |
| Lardizabalaceae | — | — | — | — | — | 1* | — | — |
| Proteaceae† | 8 ^{7†} _{1†} | — | — | — | — | — | — | — |
| Urticaceae† | 1† | — | — | — | — | — | — | — |
| Polygonaceae§ | 12 [†] _{4*} | — | — | — | — | — | — | — |
| Nymphaeaceae§ | 4* | — | — | — | — | — | — | — |
| Berberidaceae* | 8 ^{3†} _{5*} | — | — | — | — | — | — | — |
| Magnoliaceae†* | 4 ^{3†} _{1*} | — | — | — | 4†* | — | — | — |
| Anonaceae† | 3† | — | — | — | — | — | — | — |
| Myristicaceae† | 2 | — | — | — | — | — | 7 | — |
| Monimiaceae | 5† | — | — | — | — | — | — | — |
| Pedaliaceae | — | — | — | — | 1† | — | — | — |
| Lauraceae† | 6 | — | — | — | 1 | 4 | 7 | — |
| Papaveraceae* | — | — | — | 2 | 1 | — | — | — |
| Cruciferae* | — | — | — | 3 | 22 | — | — | — |
| Capparidaceae† | — | — | — | — | 1† | — | — | — |
| Resedaceae† | — | — | — | 1* | — | — | — | — |
| Moringaceae† | — | 1 | — | — | — | 1 | — | — |
| Saxifragaceae* | 1 | — | — | 1 | — | — | — | — |
| Cunoniaceae | 9† | — | — | — | — | — | — | — |
| Myrothamnaceae | — | — | 1 | — | — | — | — | — |
| Hamamelidaceae† | 1 | — | 4 ^{2†} _{2*} | — | — | — | — | — |
| Rosaceae* | 16 | 7 | — | 6 | 2 | 8 | 1 | — |
| Leguminosae§ | 123 ^{39†} _{1*} | 67 ^{12†} _{1*} | 39 ^{34†} | 4* | 23 ^{3†} | 18 ^{14*} _{4†} | 4† | — |

* Mostly temperate.

† Mostly semi-tropical.

‡ Mostly tropical.

§ Widely distributed.

TABLE I.—*Continued*

| Family | Tannin | Gum | Resin | Drying Oil | Semi-Drying Oil | Non-Drying Oil | Fat | Wax |
|-------------------|-------------------------------|-----|-------|------------|-----------------|----------------|-----|-----|
| Geraniaceae*† | 9 | — | — | — | — | — | — | — |
| Oxalidaceae*† | 1† | — | — | — | — | — | — | — |
| Tropaeolaceae† | — | — | — | — | — | 1† | — | — |
| Linaceae§ | — | — | — | — | — | — | — | 1 |
| Humiriaceae | — | — | 2† | — | — | — | — | — |
| Zygophyllaceae | — | — | 2 | — | 1 | — | — | — |
| Rutaceae†† | 1 | 2 | — | — | 5 | — | 1 | — |
| Simarubaceae† | 2 | 1 | — | — | — | 1 | 5 | — |
| Burseraceae† | — | — | 35 | — | — | 4 | 1 | — |
| Meliaceae† | 3 | 6 | — | 2 | — | 1 | 7 | — |
| Polygalaceae§ | — | — | — | — | 1 | 1 | 3 | — |
| Euphorbiaceae† | 5 | — | 1 | 8 | 6 | 1 | 1 | 4 |
| Malpighiaceae† | 4 | — | — | — | — | — | — | — |
| Coriariaceae | 3 | — | — | — | — | — | — | — |
| Anacardiaceae† | 24 | 12 | 15 | — | 1* | 2†† | 4 | — |
| Euphorbiaceae§ | — | — | 5 | — | — | — | — | — |
| Aquifoliaceae | 5 ^{3†} _{2*} | — | — | — | — | — | — | — |
| Celastraceae§ | 2 | — | — | — | 2* | 1† | — | — |
| Aceraceae* | — | — | — | — | 2* | — | — | — |
| Hippocastanaceae* | 4* | — | — | — | — | 1* | — | — |
| Sapindaceae† | 2† | — | 1† | — | — | 2* | 5† | — |
| Rhamnaceae§ | 3 ^{1*} _{2†} | — | 2† | 1* | — | — | — | — |
| Vitaceae† | 1* | — | — | — | 1* | 2* | — | — |
| Elaeagnaceae | 1† | — | — | — | — | — | — | — |
| Tiliaceae† | 3† | — | — | — | 2* | 1† | — | — |
| Bombacaceae† | — | 4† | — | — | 1† | — | 3† | 1† |
| Malvaceae | — | — | — | — | 1†* | — | — | 1† |
| Sterculiaceae† | 3† | 4† | — | — | — | 2† | 2† | — |
| Ochnaceae† | — | — | — | — | — | — | 1† | — |
| Caryocaraceae | — | — | — | — | — | — | 1† | — |
| Dilleniaceae† | 3† | — | — | — | — | — | — | — |
| Theaceae† | 4 ^{1*} _{3†} | — | — | — | — | 4† | — | — |
| Guttiferae† | 1† | — | 21† | — | — | 2† | 7† | — |
| Dipterocarpaceae† | 1† | — | 26† | — | — | — | — | — |
| Tamaricaceae† | 3 ^{1†} _{1*} | — | — | — | — | — | — | — |
| Cistaceae* | 3 ^{1†} _{1*} | — | 3* | — | — | — | — | — |
| Cochlospermaceae | — | 1 | — | — | — | — | — | — |
| Flacourtiaceae† | 1† | — | — | 1† | — | — | 5† | — |
| Cactaceae†† | — | 4† | — | — | — | — | — | — |
| Penaeaceae | — | 2† | — | — | — | — | — | — |
| Caricaceae†† | — | — | — | — | — | — | 1† | — |
| Thymelaeaceae§ | 1† | — | — | — | 1 | — | — | — |
| Sonneratiaceae | 1† | — | — | — | — | — | — | — |
| Punicaceae†† | 1† | — | — | — | — | — | — | — |
| Myrtaceae† | 26 | — | 1† | — | 2†† | 1† | — | — |
| Lecythidaceae† | 4† | — | — | — | — | — | — | — |
| Rhizophoraceae† | 11† | — | — | — | — | 1† | — | — |
| Combretaceae† | 19† | 3† | — | — | — | 1† | — | — |
| Melastomataceae† | 4† | — | — | — | — | — | — | — |
| Onagraceae* | 1 | — | — | 1* | — | — | — | — |
| Halorrhagidaceae§ | 1* | — | — | — | — | — | — | — |
| Araliaceae*† | — | 1† | 1† | — | — | 1 | — | — |

TABLE I.—Continued

| Family | Tannin | Gum | Resin | Drying Oil | Semi-Drying Oil | Non-Drying Oil | Fat | Wax |
|-------------------|-------------------------------|-----|-------------------------------|-------------------------------|---------------------------------|----------------|-----|-----|
| Umbelliferae* | — | — | 14 | — | 13*† | 1† | — | — |
| Cornaceae* | 1* | — | — | — | — | 1† | — | — |
| Ericaceae* | 10 | — | — | 3* | — | — | — | — |
| Myrsinaceae†† | 2 | — | — | — | — | — | — | — |
| Plumbaginaceae§ | 7* | — | — | — | — | — | — | — |
| Sapotaceae† | 11 | — | — | — | — | 1† | 7† | — |
| Ebenaceae† | 2† | — | — | — | 1* | — | — | — |
| Styracaceae†† | — | — | 2 ^{1†} _{1†} | — | — | — | — | — |
| Symplocaceae | 1† | — | — | — | — | — | — | — |
| Oleaceae§ | 5 ^{4†} _{1*} | — | — | — | 1† | 2†† | — | — |
| Salvadoraceae† | — | — | — | — | — | — | 2† | — |
| Loganiaceae† | — | — | — | — | — | — | 1† | — |
| Apocynaceae† | 5† | — | — | 2 | — | 2† | — | — |
| Asclepiadaceae† | — | — | — | — | — | 1† | — | 2 |
| Convolvulaceae† | — | — | 6 | — | — | — | — | — |
| Boraginaceae§ | 1* | 1† | — | — | — | — | — | — |
| Verbenaceae§ | 4† | — | — | — | — | 1† | — | — |
| Labiatae* | — | — | — | 4 ^{2†} _{1*} | — | — | — | — |
| Bignoniaceae† | 8† | — | — | — | — | — | 1† | — |
| Solanaceae† | — | — | — | 1* | 5 ^{4†} _{1*} | — | — | — |
| Scrophulariaceae§ | — | — | — | 1 | — | 1* | — | — |
| Martyniaceae† | — | — | — | — | 1* | — | — | — |
| Gesneriaceae† | 1 | — | — | — | — | — | — | — |
| Acanthaceae† | 4 ^{3†} _{1*} | — | — | — | — | — | — | — |
| Rubiaceae† | 10† | — | 6† | — | — | 1† | — | — |
| Caprifoliaceae*† | 2* | — | 1 | — | — | 1* | — | — |
| Cucurbitaceae† | — | — | — | — | 16 ^{3*} _{13†} | 2† | 2† | — |
| Compositae§ | 1 | 2† | 6 | 7†† | 2 ^{1†} _{1*} | 1† | — | 1† |

From the standpoint of ecology it is of interest to note that of the resin-containing plants, 17, or 63 percent, are of tropical habitat and 2, or 7 percent, are found in the temperate zone. This fact is also borne out by statistics of the resin plants that contain fat, as 52 percent have non-drying oils and 30 percent have drying oils. It has been shown in a previous paper by the writer (1929) that tropical plant families contain by far the greater percentage of non-drying oils or fats.

Resin is found in secretory passages and heartwood.

Function

Resin may serve as an occluding material in case of mechanical injury, may protect against animal foes, or may change the consistency of latex in plants. When secreted it may serve to protect against excessive transpiration and against animals, and to attract desirable insects (to flowers). Resin when present in the heartwood may protect against decay.

OILS

Of the families possessing gum, 8, or 42 percent, have fat; 11, or 58 percent, have non-drying oils; 7, or 37 percent, have semi-drying oils; and 3, or 16 percent, have drying oils.

Of the families possessing tannin, 17 or 21 percent, also have fat; 29, or 36 percent, have non-drying oils; 16, or 20 percent, have semi-drying oils; and 15, or 19 percent, have drying oils.

Of the families possessing resin, 7, or 26 percent, also have fat; 14, or 52 percent, have non-drying oils; 9, or 33 percent, have semi-drying oils, and 8, or 30 percent, have drying oils.

It has been shown in a previous paper by the writer that tropical plant families contain by far the greater percentage of non-drying oils or fats. Consequently, gum-, tannin-, and resin-bearing plants should be mostly tropical in habitat. Likewise gum-bearing plants should be more common in the tropics than resin- or tannin-bearing plants. This ecological conjecture agrees with the actual habitats of the plants.

The distribution of gum, tannin, resin, and the oils by families and by climate of habitat is shown in table I.

SUMMARY

The four chemical classes of gum-bearing plants may be subdivided according to their content of tannin or resin.

The seven chemical classes of resin-bearing plants may be subdivided according to their content of gum and tannin.

A plant family or genus may contain gum, tannin, or resin.

Tannin-containing families and genera are most prevalent.

Most plant families and genera that contain gum also contain tannin; a lesser number contain resin.

Few of the plant families or genera that contain tannin also contain either gum or resin.

Most plant families that contain gum also contain tannin; a lesser number contain resin. A small number of genera that contain gum also have tannin or resin, the former being more numerous.

Only four families and one genus are known to contain all three constituents.

A plant family that contains gum, tannin, or resin may also have fat, non-drying oil, semi-drying oil, or drying oil.

Most gum families have non-drying oils, while drying oils are in the minority.

Less than half the tannin families have a specific oil, non-drying oils predominate, and drying oils are least common.

Half of the resin families are associated with a specific oil, and in these non-drying oil predominates.

As indicated by the nature of their oils and also by actual location, gum, tannin, and resin families are for the most part tropical; gum families are least common in the temperate zone and tannin families most common.

Gum-bearing plant families are more common in the tropics than resin- or tannin-bearing families and gum is therefore presumably more useful to plants in the tropics than is resin or tannin.

Tannin-bearing plant families are more abundant in temperate climates than gum- or resin-bearing families and tannin is therefore presumably more useful to plants in temperate climates than is gum or resin.

The greater prevalence of tannin in temperate climates coincides with its supposed function as a protection against frost.

The fact that tannin also occurs in the tropics agrees with its accredited use as a protection against animals and fungi.

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STUDIES ON THE MYCELIUM OF *PSALLIOTA CAMPESTRIS*¹

ILLO HEIN

(Received for publication October 6, 1929)

The configuration of cells and of their contents and of cell aggregates is the visible expression of the dynamic forces which control the development of the organism. Our knowledge of the structure and energy relations of cells is based largely on the interpretation of the visible form changes through which cells and their contents pass in their integration to form tissues and organs. Generally speaking, mycologists in the past have not devoted a great deal of attention to the processes controlling the form changes of fungous cells, though the fungi appear to offer a fruitful field for observation and experimentation along these lines.

LITERATURE

I have previously (1927, 1928) reviewed the literature relating to the problems concerning the formation of pseudoparenchyma and the form responses of fungous hyphae to environmental conditions.

In the voluminous literature on the fleshy fungi, comparatively little space is devoted to studies of the mycelia. Mycologists have been occupied with the study of the fruiting stages to the neglect of the mycelial vegetative stage. The mycelium long known to be definitely connected with the carpophores was regarded, before Trog (1837) clearly pointed out the connection, as an independent plant. I shall attempt to summarize the facts in the literature regarding the problems relating to spore germination and the vegetative mycelial development of *Psalliota campestris*.

Spore Germination

The germination of the spores of the common mushroom has long been regarded as difficult of accomplishment by practical growers, and both French and American spawn manufacturers who claim to grow spawn from spores jealously guard their "secret" methods. Miss Ferguson (1902) has given an adequate historical account of the literature on the spore germination of basidiomycetous fungi but fails to mention the work of Micheli (1729), and Trog (1837). Trog was interested in showing that fungous spores do germinate and gives a brief account of the cultural practices in growing the common mushroom. Hoffman (1859) appears to be the first to have published studies on the germination of mushroom spores. According to

¹ Publication authorized by the director of the Pennsylvania Agricultural Experiment Station as technical paper no. 490.

him, the germ tubes emerge from any part of the spore. They may be from one to three in number and anastomose readily with one another even in the earliest stages. In one of Hoffman's figures (Pl. 46) germ tubes from two adjoining spores have fused very near their points of emergence.

Later studies on the germination of the spores of *Psalliota campestris* were made by LaBourdette (1861), Chevreul (1861), Hartig (1874), Eidam (1875), Constantin (1891), Repin (1897), Miss Ferguson (1902), and Duggar (1901), but their methods do not assure success when employed by others.

The Mycelium

The morphology and development of mycelium and the morphology of the strand rhizomorphs have been studied especially by Schmitz (1842), Hartig (1874), DeBary (1859), and Brefeld (1896), and the results of their observations have been summarized in DeBary (1887).

Bommer (1896) found that both the filamentous mycelium and the strands of *Agaricus campestris* may occur in the same locality when the substrate consists of compact sandy clay soil. He reported no definite organization in the strand, neither rind nor medulla were recognized and he described the peripheral region as consisting of long slender hyphae while the central parts, as he found, were made up of short stout hyphae. Bommer also gives a short morphological account of the strands and sclerotia as they occur in species of *Polyporus*, *Coprinus*, *Lentinus*, *Armellaria*, *Collybia*, *Phallus*, *Cyathus*, *Scleroderma*, *Typhula*, *Panaeolus*, and several Ascomycetes.

Brefeld's (1896) account of the mycelial strands of *Agaricus melleus* is essentially similar to that given by DeBary.

Like all previous workers, Magnus (1906) reported few observations on the early development of the strands stating simply that the vegetative mycelium in *Agaricus campestris* begins as isolated strands (presumably from spores) which spread in all directions, anastomosing and branching profusely. According to his observations, the strand is built up mostly of parallel running hyphae but the details of development are not given.

Van Bambeke (1902) found that a high degree of tissue differentiation and physiological division of labor takes place in the mycelial strands of *Lepiota meleagris*. The outer cortical layer is protective in function and consists of loosely intertwined hyphae which have lost the characteristic appearance of living cells. The cortical layer encloses the absorptive region which consists of living hyphae of similar diameter to those of the peripheral region. The central cylinder contains vascular hyphae of large diameter. The vascular hyphae also function as food storage cells and the largest ones provide receptors for excretory products as shown by the calcium oxalate crystals. Numerous crystal-like bodies which stain red with eosin, safranin, or carmine, and golden-yellow with congo red are described as occurring especially in the vascular hyphae. Van Bambeke suggested

that these bodies are probably reserve food materials, and the fact that they are abundant in the strand hyphae, less abundant in the stipe cells, and rare in the pileus favors this possibility. Van Bambeke (1910) distinguished five regions in the rhizomorph of *Lepiota meleagris* on the basis of staining reactions. With eosin and haematoxylin, the peripheral hyphae stain blue, the "zone radiare" red, the intermediate zone consisting of hyphae of large diameter, red-orange to pink, the internal zone and the medulla purple.

Falck (1909) showed that the vegetative body of species of *Merulius* consists of many different often highly specialized kinds of mycelial systems which in their structure and function are comparable with the tissues of higher plants. The mycelium according to Falck (1912) grows uniformly in all directions and is independent of the usual gravitational stimuli which in general determine the growth directions in higher plants. Strand differentiation is "backwards" and in the ultimate tissue the cells have lost their former hyphal characteristics. Three tissue elements are distinguished by Falck in the mature strand: (1) vascular hyphae with wide lumen (5-60 microns in diameter; enlargement is by swelling of hyphae and not by the breaking down of lateral adjoining walls), annular, spiral and other wall thickenings and no septa; (2) fiber hyphae with narrow lumen, no septa, thick walls and without protoplasmic contents; (3) "bildende" hyphae and transition forms between these and the former. The "bildende" hyphae are actively growing, thin walled, septate and filled with protoplasm. Of special interest are the wall thickenings described in the vascular elements. Irregularly spherical thickenings and various modifications of them attached to the wall by thin stalks which point centripetally and eccentrically from the inner surface. Simple annular and spiral thickenings, double rings, sieve plates and wart-like modifications of the cell membrane are described as frequently present in the vascular hyphae.

OBSERVATIONS

In fungus plants the inherited form of the cell is the tubular hypha. Fungous hyphae are generally believed to be especially subject to environmental determination. The forms of minute organisms and the forms of the cellular elements of larger organisms are, according to Thompson (1917) and others, governed largely by simple physical laws. It is the object of the present study to determine the physical and chemical factors which determine the orientation, position, and form transformations of the inherently tubular hyphal cells of the mycelium of the common commercial mushroom, *Psalliota campestris*.

The studies were made on material prepared by the usual fixing and staining methods as well as on fresh material. Mushrooms were grown for experimental purposes in a specially constructed house (Hein, 1929), in the cellar under the headhouse, and in an abandoned cistern at the Pennsylv-

vania State College. An abundant supply of material for study has thus always been available. Studies on the mycelium were made largely from petri dish cultures in which numerous different agar media were used. Horse dung agar, lima bean agar, prune agar, nutrient agar, and various synthetic media gave most satisfactory mycelial growth.

Spore Germination and Early Mycelial Growth

The spore of *Psalliota campestris* is similar in appearance to that of a great many other agarics. It is slightly kidney-shaped, violet brown in color, thick-walled with a small eccentrically placed spine-like projection at the basal end, the former point of attachment to the sterigma. The spore usually contains a large, conspicuous, and highly refractive globule which, as Hoffman (1860) states, on the basis of simple tests, is oil. Sometimes two or three additional very minute globules are present and frequently these are closely appressed upon each other.

In the early stages of germination the appearances are similar to those described by Repin (1897) and Miss Ferguson (1902). The germination of the spore after repeated trials was accomplished with comparative ease in a variety of different media. The spores can be germinated in distilled water, dung decoction, and various synthetic media in from 10 to 20 days. The principal difficulties are in collecting and keeping the spores free from contamination during the period preceding germination. I have frequently obtained 50 percent to 75 percent germination in distilled water after two weeks and find that ordinary bacteriological cleanliness and precautions are all that is necessary.

One, two, or even three spherical or irregularly shaped germ tubes (Pl. XII, figs. 1-3) are produced from any part but usually from the ends of the spore. I have found but few germ tubes arising from the sides of the spores (figs. 7 and 8) as shown in Miss Ferguson's figure 2, Plate 1. At this stage the germ tube is suggestive of a viscous liquid oozing out of a minute opening in the spore wall. It gradually enlarges to a bubble-like outgrowth until a diameter equal to the narrower dimension of the spore is reached when it proceeds to elongate into a typical hyphal growth.

I have never been able to observe germ pores present before the emergence of the germ tube and after it emerges the pore does not enlarge and the tube continues to remain in continuity with the spore-plasm by the very thin thread-like cytoplasmic connection.

Branching begins early (fig. 7), sometimes immediately after the germ tube is formed (fig. 2) and is far more abundant and profuse than in most fungi. The hyphae in these early stages usually have a characteristically gnarled appearance and although growth and branching are somewhat irregular the hyphae as a rule become radially oriented about the spore from the beginning (fig. 9). Septa are frequently present before branching has become profuse (fig. 6) though usually they become abundant in some-

what later stages after six or more branches have been produced. Anastomoses occur early both apically and laterally between hyphal branches from the same spore and between hyphae produced by different spores. Hyphae frequently cross each other or grow side by side with abundant fusions of the adjoining walls (figs. 11 and 13). The early stages figured on Plate XII are typical. The mycelium maintains the same general external appearance whether the fungus is grown in agar media or in compost and there is no evidence of mutual hyphal antagonism as in many other fungi, especially *Sordaria* and *Chaetomium* (Hein, 1928). While anastomoses are abundant there is little tendency to adhesion during the early stages of active growth. When the substrate under optimum moisture condition is more or less completely invaded by the mycelium the strands begin to develop.

Mycelial Strands

Some of the possible factors to be considered in the formation of the mycelial strand are adhesion, crowding, branching, air currents, carbon dioxid concentration, moisture in hygroscopic and in hydroscopic relations, chemotropism, and thigmotropism.

In petri dish cultures the strand begins to develop only after the substrate is covered with the filamentous mycelium and then it grows radially from the point of inoculation, the strands enlarging gradually from the center outward. It thus has a slightly tapering form from the beginning and generally maintains this form throughout since the increase in diameter is continuous. In this respect it is analogous to the growing point of the stems in higher plants except, of course, that enlargement is symphyogenous. There is no apical meristematic region of growth as described by DeBary (1884) for *Agaricus melleus*.

In the compost the general outward appearance of the strands is similar to that of those in petri dishes and the gnarled shapes are common in both the filamentous and strand mycelium. The growth directions in the compost are very irregular, although here too there is a general radial tendency from the point of inoculation. Under the optimum conditions of moisture, that is, when the absolute water content is between 30 and 45 percent, the compost is more or less completely invaded by the filamentous mycelium. Then the strands begin to develop. It appears from my observations that water content of the compost, whether in hygroscopic or in hydroscopic relations, is the most important factor to be considered in synnematus development. It is common to find that strand development predominates in very moist compost and that the filamentous mycelium grows best in dryer compost. There seems to be a direct relation between water content of the compost and synnematus growth. Where the compost is so moist that water can be pressed out of it by squeezing a small handful no growth occurs, but where the compost is very moist, yet no moisture can be expressed by hand, synnemata grow profusely and develop

into cylindrical strands which frequently attain diameters of from 2 to 5 millimeters. In less moist parts the mycelia tend to form strands of lesser diameters and growth is more abundant. A gradual diminution in amount of strand formation occurs in progressively less moist compost until in regions which appear almost dry the filamentous mycelial growth predominates. In the latter few strands grow and then only when the medium is completely interpenetrated by the filamentous mycelium.

The commercial mushroom grower states that where the mycelium is "stringy," that is, where a large amount of strand development takes place, a poor crop usually results. Similarly, that spawn is said to produce a poor crop in spawn bottles in which there is predominately "stringy" growth. The general opinion among growers seems to be that high temperatures produce the "stringy" mycelium but I have failed to find any relation between temperature and synnematus development. Under similar temperature conditions it has been found that in the compost both the filamentous mycelium and synnemata grow with equal profusion and that moisture content appears to be the controlling factor. The upper layers of the compost in the bed are usually dryer than the lower ones and it is common to find in the beds a gradual transition from uniform filamentous mycelial growth to more "stringy" growth lower down in the moister parts, to very sparse growth and finally none at all in the deepest very moist sections. The moisture content of the substrate is obviously determined by such factors as exposure, humidity of the atmosphere, degree of decay (which determines the porosity of the straw), and perhaps also by other factors such as colloid content.

The absolute water content of the various composts was determined by weighings from spawned beds after the substrate had become completely penetrated and it was shown that where the moisture content is above 75 percent no growth takes place; where the water content is between 60 percent and 70 percent very little but thick, stringy mycelium with little branching develops. Where the water content is between 55 percent and 65 percent some filamentous growth takes place and abundant more or less uniform profusely branched strands are produced. In composts containing between 40 percent and 50 percent water no strands appear but a uniform filamentous mycelial growth completely invades the compost to which it gives a bluish appearance. The straw particles themselves tend to be a buff to reddish yellow. In more moist compost the color varies from dark brown to almost black and in the extremely moist parts they tend to be a very dark reddish brown.

Morphology and Development of the Strand

In the early stages of strand development there is a loose aggregation of more or less parallel growing hyphae. Branches tend to form acute angles with the developing strand though frequently there are branches which

form right angles to the main axis of growth (fig. 14 *a*). There does not seem to be an inherited definite angle of branching as sometimes appears to be the case in other fungi (Hein, 1928), for even though the branches are frequently free to grow in any direction so far as mechanical barriers are concerned, both right and acute angles are formed. Many branches from the young strand form very sharp angles and follow the growth directions of the main bundle of hyphae, thus contributing to the strand enlargement. In such cases it is possible that a slight adhesive tendency or possibly positive chemotropic or other stimuli tend to hold the branch to the developing strand. That the adhesive tendency is very slight or that possibly it does not exist is shown by the intermittent contacts and generally loose hyphal aggregation as shown in figure 14.

Appearances like those shown in figure 11 are typical of the earlier stages in hyphal differentiation. Numerous branches which anastomose irregularly both laterally and at the hyphal ends fill in intervening space, building up a cylindrical plectenchyma. Where two or more hyphae fuse by the dissolution of their lateral adjoining walls as in figures 12 and 13 a cell of large diameter results. In this manner the vascular elements of large diameter originate. The large lumen in the vascular hyphae of *Psalliota campestris* does not appear to be entirely the result of swelling as Falck described for species of *Merulius* (1912) but is at least partly the result of the lateral fusion of hyphae of smaller diameters. Continued branching and growth from the older portions surround the earlier developed parts so that they come to lie in the center. There is thus a peripheral accumulation of mycelium resulting in an increase in strand diameter. The youngest parts will, therefore, be on the outside with increasingly older parts towards the center.

Figure 14 represents the growing tip of an older strand. The hyphae are all of uniform diameter, filled with protoplasm and actively growing. These are the "Bildungshyphen" of Falck (1912). The latter hyphae will become differentiated by lateral fusion into vascular hyphae of large diameters. The end-to-end fusion by dissolution of the intervening septa results in the formation of the long tubes. Eventually hyphae such as are represented in figure 14 will become surrounded by the younger ones which in turn will become similarly enveloped until the strand is completed. There appears to be no definite inherited limit to the increase in strand diameter. Fruiting may take place on strands of narrow diameter as well as on those of wide diameter, hence size appears to be no index to maturity. The diameter of the strand is evidently dependent entirely on environmental conditions and may vary from a fraction of a millimeter to 5 or more millimeters.

The further differentiation of the strand elements would seem to be largely the result of contacts and pressures, some inflation, and further fusions preceded by gelatinization of lateral and end walls at the places of fusion.

Differentiation in the strand begins early at stages when a diameter of between one-fourth and one-half a millimeter has been reached. In transverse section (Pl. XIII, fig. 25) such young strands will show two poorly defined regions: a peripheral zone of uniform slender hyphae, repeatedly bent in various directions but with a general centripetal tendency; and a more compact cylindrical bundle of parallel hyphae of various but mostly large diameters. The former are rich in cytoplasm, contain a few small granules, and the septa are provided with the protoplasmic connecting pads to be described below. In the central area the hyphal cells of large diameter become progressively smaller toward the periphery. Slender hyphae similar in appearance to the peripheral ones are irregularly distributed throughout the strand but they become progressively more numerous toward the outside, finally making a somewhat continuous but irregular, fairly compact layer of variable thickness (fig. 25 *c*). The large central hyphal cells no longer have the typical cylindrical shapes but have become somewhat flattened on their adjoining surfaces of contact (fig. 25).

In older strands differentiation becomes more marked. The surface appears under the lens as a loosely tangled mass of uniform, branched, slender hyphae which appear to have no specific directions of growth (figs. 15 and 16). In radial section such a strand shows two sharply defined regions with distinct staining reactions. A densely stained (blue, with the Flemming triple) central region is surrounded by a peripheral zone which shows a greater affinity for the red dye. The latter zone consists roughly of two indistinctly defined layers. In the outermost the hyphae are similar in every respect to those described above in the young strand and enclose a loosely interwoven prosenchyma in which the hyphae tend to be parallel to the main axis of the strand.

In the transverse section (fig. 27) the cells of large diameter in the core region show considerable difference in shape from those in similar young stages. The shapes are often quite remarkable. In general appearance the section is suggestive of children's mosaic puzzle pictures, the various different cells fitting in closely together, leaving no spaces. A compact mass of numerous small cells, circular in outline, and less numerous, large, irregularly shaped cells make up the picture. The large cells are similar in transverse section to the outline of many of the abnormal lily nuclei figured by Miss Goldstein (1928); lobed, amoeboid lobulate, horned, stellate with rounded corners, bullate, and various combinations of these as well as oval and circular forms may be found. The particular form at a given point is not at all constant throughout the length of the cell but varies continuously even at short distances, as can be seen by focusing up and down through a thick section.

The longitudinal sections, too, show that the cells are extremely irregular in shape. The shape depends on the number of cells which have fused,

the completeness of fusion, whether throughout its length or only at few places (figs. 33-35), the mechanical resistance offered by surrounding hyphae, the amount of expansion, and perhaps other factors. That there is some expansion of these cells is indicated by the plump appearance, the dense crowded condition, and the general compactness with filling in of all interhyphal spaces.

By tracing in a section a single filament from a strand the variation in diameter and form may be noted. A single hypha of large diameter will often be fused laterally at several places with other similar hyphae (figs. 33-35) as well as with several hyphal cells of small diameters. The lateral combination of a large cell with one or more smaller ones is partly the cause of the odd shapes described above in the transverse section. Such cells as are shown in figures 33, 34, and 35 are never continuous with similar large cells over any great distance but are joined end to end by hyphae of small diameters. At times there is a gradual tapering to the smaller cell (fig. 34 *c*) without interruption by septa. Since in early stages septa occur with greater frequency than is shown in either figures 34 *c* or 35, it is probable that the septa have been resorbed. In most cases, however, a septum is present between the small and large cell (figs. 33 *a* and 35 *a*). The general appearance of parts of all the cells of large diameter indicate that they have become expanded. The upper part of the cell in figure 32 with its bulb-like enlargement certainly owes its form to expansion.

That the large cells probably function as conductive vessels is indicated by their large size, the absence of numerous septa, the perforations usually present in the septa when they do exist, and the paucity of cytoplasmic contents if any. Such appearances as shown in figure 34 *b* where the stained material appears to be moving through the pore in the septum suggest movement of materials from cell to cell. The occasional appearance of thread-like cytoplasm especially near the pore in the septum as in figures 39 and 33 *d* and *e* also indicates a flowage of materials, yet the latter appearances are not common.

Very often where a large cell tapers to narrow diameter the cytoplasm will be very dense but smooth, as in figure 34 *a*, in which there is an apparent congested condition due possibly to the abrupt narrowing of the diameter. Then too where two cells of large diameter are constricted at the septum as in 35 *c* there frequently is a dense accumulation of cytoplasm.

Occasionally large irregularly shaped cells are found in older strands. Figure 36 shows a large cell which appears as though it had branched laterally and subsequently expanded. The blind end has no connection with other hyphae and appears either to have grown between the surrounding slender filaments or to have become overgrown by them. Such spherical bodies as are figured on the left side of the cell in figure 36, as well as the two spheres in the connecting hypha and the larger irregular ovoid

bodies at the pore in the septum between the two large cells, in many cases remained unstained with the dyes used. These are yellow in color, highly refractive, and may possibly be oil.

In more deeply stained sections the bodies will stain pale blue but then only in certain cells, and not all cells will show them to be equally stained. From their different staining reactions and the fact that they are well rounded, usually quite spherical, I am of the opinion that they are not of the same chemical composition as the deeply stained bodies described in figures 36, 33, 34, and 35. The latter tend to have plane faces and are always deeply stained.

In many places the strand exhibits masses of short cells similar to those figured in 38. These are doubtless strands running at right angles or nearly so to the plane of section which have become overgrown by the strand tissue running in the direction shown in the figure. They may be compared with the knots found in woody plants, older branches which have become surrounded with the accumulated growth of younger tissues.

The main cylinder of hyphae consists mostly of slightly thicker walled cells of large diameter and numerous cells of all smaller sizes down to slender cells similar to the peripheral ones. There is generally an outer, thin, compact layer of slender hyphae somewhat like the above except that many have fusiform shapes, and in section often show the typically circular outline to be modified through crowding. Many of the cells of large diameter are similar in appearance to the vascular hyphae described by Falck for *Merulius* and *Lenzites* (1909 and 1912) and are doubtless similar in function. These are in general progressively larger toward the center although various sizes can be found throughout the section (fig. 36 *b* to *d*). Numerous slender hyphae containing dense, irregular shaped granules and little cytoplasm may be found in all parts of the strand (fig. 26 *e*). They are similar in appearance to the fiber hyphae described by Falck (1912).

Many of the larger vascular hyphae suggest by their plump, inflated appearance, well rounded at the septa (fig. 26 *c*, *f*, and *g*), that they have become distended. Increase in diameter thus appears to be in part due in this species to swelling through internal pressure as described by Falck (1912) for species of *Lenzites*. That the large size of the vascular hyphae is also brought about by the lateral as well as end-to-end fusion of two or more smaller hyphal cells is indicated by the gelatinized cell walls and cell walls in a partial state of disintegration. Figures 21 and 22 show gelatinized septa which have opened at the center. It appears that the septa become gelatinized and slowly dissolve away in some cases, whereas in others (figs. 23, 24, and 33), no gelatinization takes place but the walls become laminated and dissolve away. Possibly in the latter case the apparent tearing is a consequence of lateral expansion of the cell, resulting in a tearing of the septum which is later dissolved. Figure 33 shows the septum to be almost completely dissolved away leaving only an annular thickening

on the wall. The ringed and spiral thickenings described by Falck (1912) may possibly be the remains of septa. The peculiarly shaped "Balken" of Falck, too, may be the remains of such septa. Several of his figures are similar to 21 and 22 but the latter thickenings are annular.

That there may be lateral fusion of hyphae in older strands is shown by the frequent appearance of irregular incomplete cell walls (fig. 26 *a*), frequent broken walls (figs. 26 *d*, 23, and 24), and various other appearances which suggest this possibility. Where a very wide cell meets end to end with several of smaller diameters as at figure 35 *b* and 26 *i*, it would appear that the upper cell has been formed by the fusion of several smaller hyphae while those below have remained slender. Of the latter, the walls near cell *i*, figure 26, have been partly dissolved away. Presumably these will eventually become hyphae of large diameters.

Crystalline Incrustations

The mycelium in the substrate under low magnifications presents a diffused appearance because of the more or less complete covering by needle-like and other crystals. All single hyphae as well as synnemata and strands are completely covered with them. The crystals vary in length and arrangement on the mycelium where they may be attached by their ends or loosely at any point. They present a disorderly scattered appearance over the hyphal surface.

In size the crystals are extremely variable. They may be very short, 1 to 2 microns (figs. 27 and 28), or extremely long, 15 to 20 microns (figs. 29 and 30) and vary in breadth from less than a micron to two or three microns (fig. 31). In general they are needle-like and of varying geometrical patterns in transverse section but here and there, usually on older hyphae, short, broad, angular crystals of varying shapes may be found (fig. 31). All react to the usual tests for calcium oxalate.

Hemispherical Pads

In the living hyphal cells as well as in prepared material the hemispherical pads mentioned by Strasburger (1884), Harper (1902), and Levine (1913) for other basidiomycetous fungi, are present, one on each side of the center of the septum. The pads stain a deep red with safranin, are very variable in shape (but always rounded) and size, and are connected with one another by a very thin thread of similarly stained material which apparently passes through a minute pore in the center of the septum. Occasionally there are pads almost spherical in form (fig. 17) and others are concavo-convex (fig. 19), but ordinarily they are hemispheres. Sometimes there is a very large one on one side of the septum attached to a small one on the other side (figs. 17 and 18) and frequently extremely small pads may be found (fig. 20).

There appears to be no correlation between the presumed general

direction of cytoplasmic movement from one hyphal cell to another and the position of the large and small pads. A large pad may be on the distal end of a septum while a small pad may be opposite it in the proximal end of the adjoining cell or vice versa.

DISCUSSION

All fungous tissues originate in the cylindrical hypha. The further growth and differentiation into the various kinds of fungous tissue are the result of environmental responses which vary in kind and degree during the stages of development. In the mycelium of *Psalliotia campestris* only two possible factors appear to be inherited, the tubular form and the tendency to anastomose. Yet even these factors may be regarded partly, at least, as environmental responses. When the germ tube emerges it is spherical in form. Its protoplasm is undoubtedly, from its appearance and behavior, liquid or semi-liquid at this stage and is, therefore, subject to surface tension forces. The germ tube or rather germ vesicle when it reaches a size equal to the narrower diameter of the spore, about 4 microns, immediately proceeds to elongate to a tubular hypha which is of fairly constant diameter. Whether the limit in size is to be regarded as inherited or as an environmental response is to be considered. The diameter of the germ tube may be thought of as limited by the elasticity of the hyphal membrane. Immediately after emergence the wall may be more nearly liquid and elastic but after exposure to the atmosphere or to the substrate, chemical or physical changes, possibly water loss, may take place which render it less so. Then a certain balance between the internal cytoplasmic pressure and the elasticity of the hyphal wall determine the diameter attained. Beyond a certain point in the elasticity of the enclosing membrane the internal pressure may not be able to overcome its resistance. The hypha is youngest at the tip and here internal pressure may be thought of as constantly pushing forward the thin, less solid and hence more plastic tip until the hypha reaches a diameter determined by the degree of elasticity of the wall.

Little adhesion has been noted in the formation of the strand. Parallel hyphal aggregation with anastomoses and constant filling in through growth with branching results in the strand tissue.

That a greater amount of strand development takes place in moist compost than in relatively dry substrate as reported above suggests the possibility that hyphal fusions are the most important controlling factors in strand formation. Hyphal fusions may be more abundant under moist than under dryer conditions. Functionally it would appear that the breaking down of the hyphal cell walls are an adaptation to facilitate the movement of materials from cell to cell and for the development from hyphae of small diameters to vascular hyphae of large capacity.

When the strand has reached a stage of dense hyphal growth, further

intercalary growth and branching results in crowding to such a degree that there are developed mutual lateral pressures which result in the modification of the cylindrical form, as has been described. If the hyphal cells were all of fairly uniform size we should expect the usual hexagonal outline of the cells in transverse section but this does not occur. There is no uniformity in size, hence every imaginable shape is the result (fig. 25).

There can be no question that the cells of large diameters owe their size both to lateral fusion with resorption of the adjoining walls, and to expansion. That this enlargement is a functional adaptation to food storage is possible. Presumably food materials are stored in the large cells of the strand preliminary to the production of carpophores. The largest cells may, therefore, be regarded as food reservoirs. The hyphal tubes as they become filled with liquid food expand as shown in figures 32 to 35 and the pressure so developed may possibly assist in the movement of the contents in later stages when the developing carpophores rapidly use up the stored materials.

The hemispherical pads at the end walls of the hyphal cells, from their appearance, suggest that they are spherical masses of cell products in process of translocation from cell to cell. They appear to be a viscous liquid, penetrating the pore in the septum. The frequent appearance of a small hemispherical or spherical body on one side and a larger one on the other side of the septum and similarly stained globules of various sizes commonly near the septum (fig. 36), though often in any part of the cell, suggest that they may be, in this fungus at least, movable cell products and not connecting pads. In some preparations these bodies are yellow in color showing no affinity for the dyes used. In figure 36 there is no connection between the two bodies on each side of the septum and they appear as though they are the halves of a larger globule separated in the process of passing through the pore.

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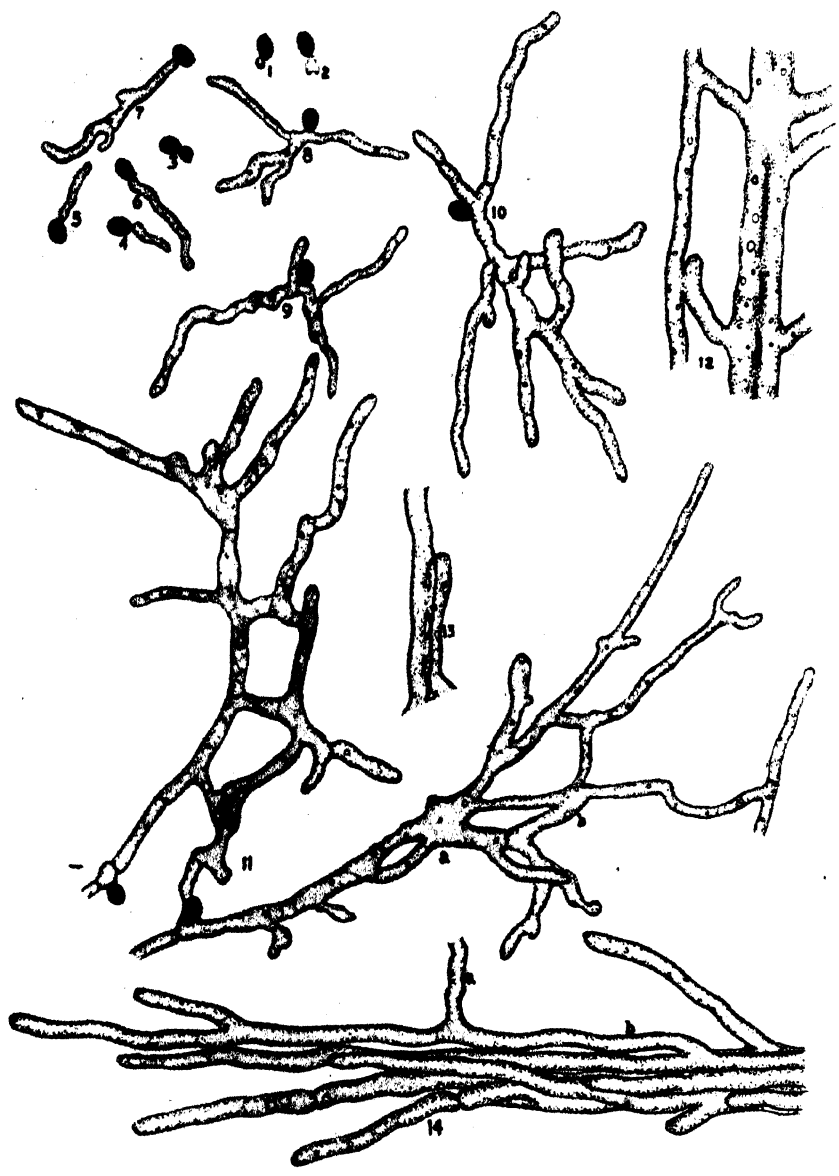
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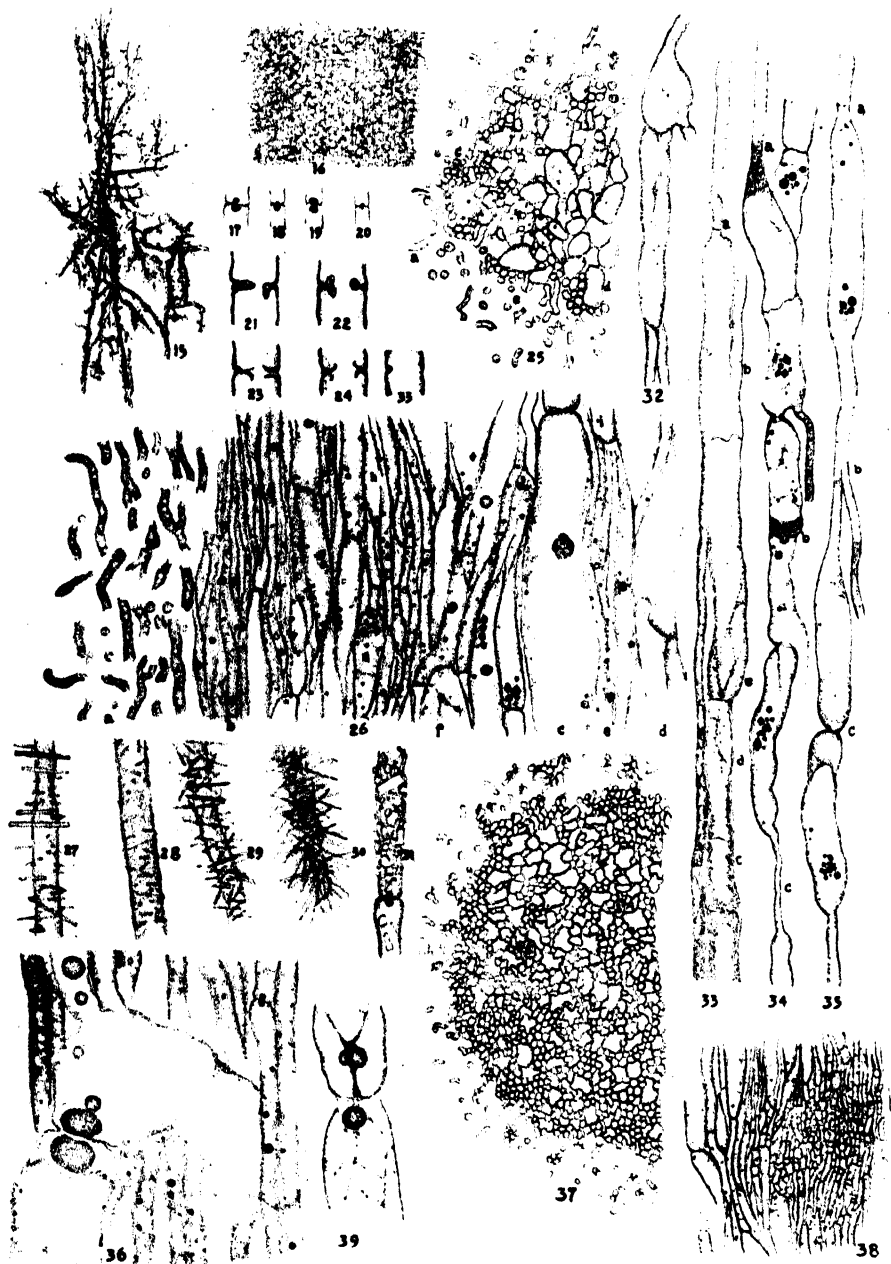
EXPLANATION OF PLATES

PLATE XII

Drawings were made with the aid of the Abbe Camera Lucida. Figures 1 to 15, 17 to 20, are magnified 600 diameters; figures 25, 26, 32 to 35, 37 and 38 are magnified 300



HEIN: PSALLIOTA



HEIN: PSALLIOTA

diameters; figures 21 to 24, 27 to 31, 33, 36, 39 are magnified 600 diameters; figure 15 is natural size and 16 is magnified 8 diameters.

FIG. 1. Early stage in spore germination showing spherical germ tube.

FIGS. 2 and 3. Slightly later stages with irregularly shaped germ tubes.

FIGS. 4-6. The germ tubes have elongated to typical hyphal shapes.

FIG. 7. Lateral branching in earliest stage.

FIGS. 8-10. The branches have become radially oriented.

FIG. 11. Well-developed hyphal system with numerous, both lateral and end-to-end fusions.

FIGS. 12 and 13. Lateral fusion of hyphae.

FIG. 14. Aggregation of hyphae forming the growing point of the strand.

PLATE XIII

FIG. 15. Habit sketch of mycelium from compost.

FIG. 16. Surface view of short piece of the strand.

FIGS. 17-20. Hemispherical pads.

FIGS. 21-24, and 33. Stages in the dissolution of the septum. 21 and 22 show the gelatinization of the septa.

FIG. 25. Transverse section of one half of a young strand.

FIG. 26. One half of a radial section through an older strand.

FIGS. 27-31. Various kinds of crystalline incrustations on the hyphae.

FIGS. 32-35. Enlarged hyphal cells from the strand tissue.

FIG. 36. An irregularly shaped expanded hyphal cell with blind end.

FIG. 37. Transverse section through one half of an older strand.

FIG. 38. Strand in transverse section overgrown with strand tissue running at right angles to it.

TIME AND TEMPERATURE FACTORS IN HARDENING PLANTS

R. B. HARVEY

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It has been the practice in measurements of low temperature resistance to expose plants to continuous low temperatures¹ for a certain number of days and then to determine their hardiness by freezing them at constant temperatures considerably below the freezing point of the tissues. By making several tests at a series of freezing temperatures, comparisons can be made between the relative hardening capacity of varieties and also of the rate of acquiring hardiness. If a single uniform variety of plant is used, a comparison can be made also between the effectiveness of different treatments for hardening plants.

In testing the relative effectiveness of various temperatures and time exposures in evoking hardiness, cabbage plants were used because they can be hardened easily, the evidences of freezing injury are readily seen, and varieties with known and relatively uniform hardening capacity are available. The plants were exposed at 20° C., 10° C., 5° C., and 0° C. for five days continuously, and then the hardiness was determined by freezing for twelve hours at various constant temperatures, - 4° C., - 5° C., and - 7° C. When the critical freezing temperature had been determined, it was used for the test and the relative amount of injury after freezing at this temperature was recorded and used in photographs, because this injury criterion gives a more accurate comparison between treatments than the determination of the freezing temperature which is just sufficiently low to give one hundred percent killing of the plants. Furthermore, the critical killing point requires much more experimentation for its accurate determination.

Early Jersey Wakefield cabbages were grown to a height of about four inches in four-inch pots. They showed good uniformity in size and rate of growth; they also gave uniform performance in hardening trials. Five plants were taken for each exposure treatment at 20°, 10°, 5°, and 0° C. continuously for five days. Also similar sets of cabbages were alternated between various high temperatures and 0° C., being kept twelve hours at 0° C. and twelve hours at the higher temperatures, 10° and 20° C. This alternation of the temperature exposure of the plants was accomplished by moving the plants between rooms kept constantly at 0°, 5°, 10°, and 20° C.

¹ Harvey, R. B. Hardening process in plants and developments from frost injury. *Jour. Agr. Res.* 15: 83. 1918.

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The rooms were refrigerated by ammonia expansion coils. The temperature was controlled electrically and recorded by a Leeds and Northrup recording potentiometer, using resistance thermometers. The variation from the desired temperature was less than $\pm 1^{\circ}\text{C}$. Exactly the same number of hours exposure was given at 0°C . and each of the higher temperatures in this series. During the hardening period the plants were exposed to a light intensity which had been found sufficient to give good growth, and therefore the plants were not etiolated at the end of the hardening period. Obviously, if plants are started with the first twelve hours at the higher temperature, at the end of the hardening period they will have had twelve hours exposure to low temperature immediately before freezing. Since it was thought possible that this last period might be of some importance in determining the condition of hardiness at the time of testing, an alternate series was set up beginning at the lower temperature and ending with a twelve-hour period of higher temperature exposure immediately previous to testing.

The plants from the continuous exposures at 0° , 10° , and 20°C . were frozen together with those which had been alternated between 0 – 20°C . and 0 – 10°C . The temperature found best for showing differences in hardiness was -5°C . The exposure to this temperature was twelve hours. Evidently the threshold value for producing hardiness lies at about 5°C . for these cabbages. The results of this experiment are shown in text figure 1. Exposures at 5°C . continuously and at 0° and 10°C . at alternate twelve-hour intervals give the same product of temperature and time. Also alternate equal time exposure at 0° and 20°C . give the same temperature-time product as a continuous exposure at 10°C ., but the effect on the production of hardiness in these cases is vastly different. Cabbages exposed at 0 – 20°C . (text fig. 1, no. 5) alternately each day are much hardier than plants exposed at 10° (text fig. 1, no. 4) continuously, and those exposed for twelve hours at 0 – 10°C . (text fig. 1, no. 3) alternately, are hardier than those exposed at 5°C . (text fig. 1, no. 2) continuously; in fact, the hardiness of the plants alternated between 0° and 20°C . more closely resembles the hardiness of plants exposed at 0° (text fig. 1, no. 1) continuously than those exposed at 10° (text fig. 1, no. 4) continuously. The alternate lot ending with high temperature exposure showed no differences in injury from this lot. Evidently it is unimportant at which temperature, higher or lower, the hardening period ends. The injury to the plants kept at 20°C . continuously is shown in text figure 1, no. 6. Each of these plants in text figure 1 was selected as the average injury shown by the whole lot of Early Jersey Wakefield plants represented by them. This same experiment was repeated with three other varieties of cabbages of nearly the same age as the Early Jersey Wakefield lot, namely, Charleston Wakefield, Red Rock, and Copenhagen Market. The results of these trials checked well with the results of the first experiment. Evidently the varietal differences caused



TEXT FIG. 1. Early Jersey Wakefield cabbages. Explanation in text.

minor differences in the amount of injury, and the effect of alternating the temperature with one-half time at 0° C. was about the same in all of these varieties. Comparison of the different treatments on different varieties may give different injuries throughout the series, but the placing of each hardening treatment was maintained in different varietal series.

Since the exposure to 0° for half of the day by far overbalanced the exposure at 20° C. and 10° C., the effect of the time of exposure at 0° C. was next determined.

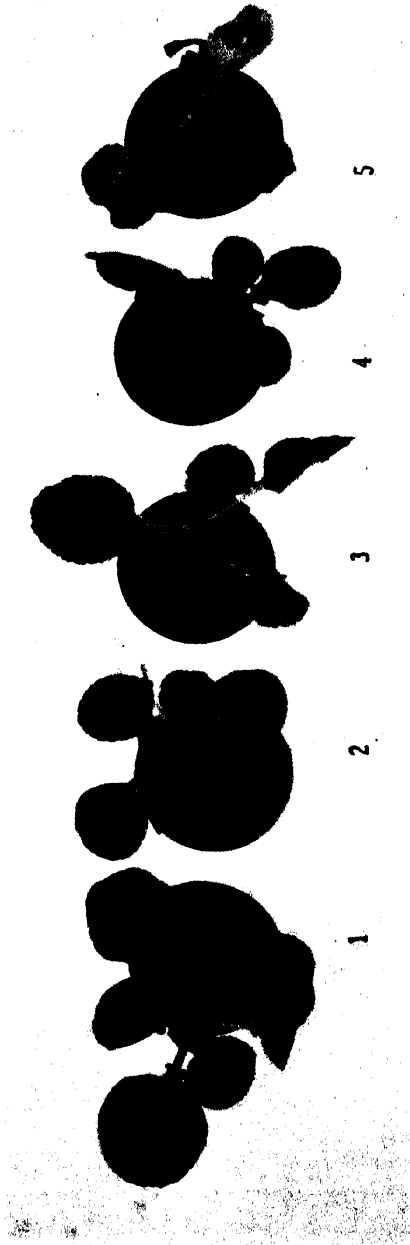
Six lots of five plants each of Copenhagen Market cabbages were given exposures as follows:

| Number of Lot in Text Figure 2 | Exposure | Hours Per Day |
|-----------------------------------|-----------------|---------------|
| 1..... | 0° C. | 24 |
| 2..... | 20° C. | 20 |
| | 0° C. | 4 |
| 3..... | 20° C. | 22 |
| | 0° C. | 2 |
| 4..... | 20° C. | 23 |
| | 0° C. | 1 |
| 5..... | 20° C. | 24 |

All of these lots were exposed on five consecutive days at the temperatures specified. They were then frozen at -5° C. since this temperature had been found to be critical for the indication of differences in the hardiness. They were then allowed to stand in a constant temperature greenhouse at 65° F. The results of this freezing trial are shown in text figure 2, photographed after the extent of the injury had become evident. Each plant represents the average injury to five plants with the same exposure.

The complete killing of the lot exposed continuously at 20° C. is shown by Lot 5 at the right. Lot 4, which had only one hour per day at 0° C. and twenty-three hours at 20° C., showed killing in the younger leaves of all but the midrib. However, the older leaves were injured only at the margins. Evidently one hour's exposure per day at 0° caused an increase of hardiness in five days which became evident in a considerably decreased injury from freezing. All plants in Lot 5 died; all those in Lot 4 survived, with severe injury only to the bud leaves. Lot 3, which received two hours per day exposure at 0° C., showed less injury than Lot 4. The older leaves in Lot 3 were not injured as much as those in Lot 4. Lot 2, exposed for only four hours per day, showed no injury at all at -5° C. just the same as Lot 1 which was exposed at 0° C. continuously.

Evidently a short exposure of one to four hours per day at 0° C. was sufficient to overbalance the effect of twenty-three to twenty hours per day exposure to 20° C. and to cause the hardening of the plants to a sufficient degree to allow them to survive exposure to -5° C. Four hours exposure each day at 0° C. caused the plants to harden sufficiently within five days to be injured no more at -5° C. than plants exposed continuously to 0° C.



TEXT FIG. 2. Copenhagen Market cabbages. Explanation in text.

The remarkable effect of so short an exposure as one hour per day at 0° might lead one to believe that a low temperature shock was sufficient to harden plants, and that hardening is a cold shock response, not correlated with the product of temperature and time exposure. If this is true, it would be in keeping with the common observation on plants growing outdoors in spring and autumn which remain resistant to freezing although the temperature may sink to near the freezing point for only a few hours at night. The differences in times and temperatures required to keep different varieties in the hardened condition will need further elucidation, but the data here presented are quite suggestive of the conditions underlying the gain or loss of hardiness under the usual day and night fluctuations in temperature.

SUMMARY

The hardiness of cabbages was determined after exposure to various continuous hardening treatments and after alternation between high and low temperatures. The threshold value for producing hardiness in Early Jersey Wakefield cabbage was found at about $+5^{\circ}$ C. Alternate equal exposures, twelve hours at 0° and twelve hours at 10° or twelve hours at 20° , produces greater hardiness than exposure continuously at the average of these temperature exposures: 5° C. in the first and 10° in the second case. The effect of short exposure, one to four hours per day, at 0° C. overbalances the effect of longer exposures, twenty-three to twenty hours per day, at 10° and 20° C.

It is suggested that hardiness in plants is a cold shock response.

UNIVERSITY OF MINNESOTA

FASCIATION OF SWEET PEAS

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(Received for publication October 18, 1929)

Since the discovery by Smith and Townsend (12) that crown gall was caused by *Pseudomonas tumefaciens*, the tendency has been to attribute the development of a majority of overgrowths, not known to be due to other causes, to infection by this pathogen. It is true that various types of overgrowths and abnormalities are caused by the crown gall organism. Examples of such are: the fleshy form of hairy root on apple (11), hairy root on sugar beet (10), the leafy crown gall of tobacco and geranium (13), and the witches broom of carnation (14). Küster (3), by inoculation with *Ps. tumefaciens*, also induced leafy crown galls and chimeras on *Pelargonium* sp.; and bifurcated trichomes and leaves, ascidia, and a thallus-like leaf petiole on *Taraxacum officinale*. Smith (14, 15, 16) was able to induce fasciation by inoculations into the leaf axils of a number of plants including *Pelargonium* sp., *Nicotiana* sp., *Ricinus communis*, *Tropaeolum majus*, and *Brassica oleracea*. Recently Miss Brown (1) isolated a weak or specialized strain of *Pseudomonas tumefaciens* from fasciated sweet peas which reproduced fasciation upon this host.

That there are non-infectious overgrowths similar in appearance to crown gall on apple nursery stock has been shown by Melhus (4), Riker and Keitt (9), and Muncie (6). Certain types of fasciations also have been proved to be due to causes other than infection by *Ps. tumefaciens*. In this connection it is interesting to note that Molliard (5), Peyritsch (8), and Miss Knox (2) induced fasciations, stem bifurcations, and rosettes in species of *Valeriana*, *Oenothera*, and other plants by means of insect injury. Smith (17) also reports a case of fasciation in *Dahlia* in which the abnormality is attributed to the action of ammonia and other bacterial by-products. In this case, the main axis of the plant was injured, and within
* the cavity there appeared a mixed bacterial growth.

The abundance of fasciations on sweet peas in the local greenhouses each winter coupled with the fact that a strain of *Ps. tumefaciens* which would induce the abnormality on sweet peas had been isolated by Miss Brown (1), led to attempts to recover this strain of the crown-gall pathogen for comparison with those already isolated from other hosts, including the apple. In this paper are presented the results of such isolation trials, and inoculation experiments with the organisms recovered from fasciated sweet peas as well as with certain non-pathogenic bacteria isolated from overgrowths on apple. Attempts also were made to induce fasciation by means of greenhouse insects. Experiments were carried out to determine the

effect of mechanical injury and environmental conditions upon the development of fasciation.

PREVALENCE AND VARIETAL SUSCEPTIBILITY

So far as observed, this abnormality on the sweet pea occurs only in the greenhouse. In greenhouse practice, sweet peas are often started by planting three or four seeds in a three-inch pot. Under these conditions, fasciation may appear upon the young potted plants when they are 12 to 18 inches in height.

The fasciated parts continue to develop after the young plants are transplanted to the permanent bed. Observations to date show that although stunting of the main shoot of the fasciated plants may occur, this condition is rarely associated with the abnormality under greenhouse conditions. Plants bearing conspicuous fasciations have been observed to produce normal growth of stems, leaves and flowers.

At first it was thought that certain varieties might show a greater tendency toward fasciation than others, but in experimental plantings of disinfected seed of several varieties in steamed soil, this did not hold true.

In the greenhouse where fasciated plants were first found, counts were made of the affected plants of several varieties in replication, growing in parallel rows. These data are summarized in table 1.

TABLE 1. *Occurrence of Fasciation on Sweet Pea Varieties in the Greenhouse*

| Variety | Percent Fasciated | Variety | Percent Fasciated |
|----------------------|-------------------|-------------------------|-------------------|
| Ball Rose..... | 26 | Snowstorm Improved..... | 55 |
| Eldorado..... | 27 | Lavender King..... | 48 |
| Zvolanek's Rose..... | 44 | Louise Gude..... | 72 |
| Yarrowa..... | 34 | Vulcan..... | 40 |

Although counts were not made of the numbers affected, several rows of the variety Spencers Mixed also showed a high percentage of fasciated plants. The phenomenon of fasciation has been observed for three years in the same greenhouse on each crop, including the varieties Zvolanek's Rose, Snowstorm Improved, Yarrowa, Mrs. Cuthbertson, Vulcan, Lavender King, Spencer's Mixed, Louise Gude, and Eldorado.

ISOLATION AND INOCULATION TRIALS

Attempts to obtain various strains of *Pseudomonas tumefaciens* for use in studies of the apple crown gall problem led to isolation trials on fasciated sweet pea plants. The first isolations which were made from a plant of the variety Mrs. Cuthbertson showed a conspicuous fasciation (Pl. XIV A). This and other plants (Pl. XIV B) of the same variety were found in the greenhouse of the Department of Horticulture in March, 1927.

Upon close examination a distinct brown lesion about two inches in

length was also noted on the main root just below the fasciation. The presence of this lesion suggested that the malformation might be caused indirectly by the organism in the lesion. Such a phenomenon is commonly seen in the production of aerial tubers on the potato as a result of infection by *Rhizoctonia Solani* B. & C. on the lower portion of the stem.

Portions of the lesion on the affected root were used in making 89 tissue plantings in potato dextrose agar. Of these 18 showed fungus growth, mostly *Fusaria*; from 48 plantings, bacterial colonies unlike *Ps. tumefaciens* in plate cultures developed; and in the remaining 23 plates, neither fungi nor bacteria developed. Cultures of the various fungi were made and employed in four series of inoculation trials upon sweet and garden peas. In these trials, the plants were grown from disinfected seed in four-inch pots of sterilized soil, in the greenhouse. An average of 104 days elapsed between the date of planting and the final records of the number of fasciated plants.

The combined results of the four series of inoculation trials upon sweet peas are presented in table 2.

TABLE 2. *Results of Inoculating Sweet Peas with Fungus Cultures Taken from a Lesion on Fasciated Plant*

| Organism | Number Plants Dug | Number Plants with Fasciation | Percent with Fasciation |
|---------------------------------|-------------------------|-------------------------------------|-------------------------------|
| <i>Fusarium</i> (1)..... | 39 | 13 | 33.3 |
| " (2)..... | 42 | 15 | 35.7 |
| " (3)..... | 24 | 3 | 12.5 |
| " (4)..... | 40 | 14 | 35.0 |
| " (5)..... | 39 | 9 | 23.0 |
| " (7)..... | 23 | 7 | 30.4 |
| <i>Ps. tumefaciens</i> | 25 | 2 | 8.0 |
| Check | | | |
| <i>Rhizoctonia Solani</i> | 37 | 12 | 32.4 |
| Check | | | |
| Sterile puncture..... | 45 | 14 | 31.1 |

In a like manner, 72 garden peas, grown in pots of sterilized soil, from disinfected seed, were inoculated with transfers of the same cultures as those used with the sweet peas. Thirteen plants were left as checks. Although the plants grew to maturity, no fasciations occurred upon any of the garden peas in these trials.

From the data in table 2, it is obvious that the percentage of fasciated plants in the uninoculated checks was as great as the average (29.46 percent) for those inoculated with the species of *Fusaria*. The plants inoculated with *Rhizoctonia Solani* showed a higher percentage of fasciation (32.4) than the average of those inoculated with *Fusaria* (29.46), and those inoculated with *Pseudomonas tumefaciens* (raspberry strain) less than either of the above (8.0), perhaps because of the small number of plants involved. The results of the inoculations with *Rhizoctonia Solani* and the

crown gall organism are not so significant. The combined data, however, suggest that none of the organisms tried are entirely responsible for the production of fasciation on the sweet pea plants.

Miss Brown (1) isolated a bacterium from the basal portion of a fasciation on sweet peas, and upon reinoculation with this bacterium, produced fasciation upon four of the eleven plants involved. She concludes that the fasciation was induced by the organism isolated, a weak or specialized strain of *Pseudomonas tumefaciens*. During the course of isolation trials from overgrowths on apple nursery stock (6) bacterial organisms having the appearance of the crown gall pathogen were obtained in the poured plates. In later studies by Patel (7) these were found to be non-pathogenic upon *Lycopersicon esculentum*, *Bryophyllum calycinum*, *Nerium oleander*, *Pyrus Malus*, *Pisum sativum*, and *Ricinus communis*.

Attempts, therefore, were made to induce fasciation upon sweet peas and garden peas grown from disinfected seed, in sterilized soil, by inoculation with 12 such non-pathogenic organisms and a culture of *Pseudomonas tumefaciens* taken from raspberry crown gall. After inoculation the plants were kept three days, without watering, in a moist chamber. They were then arranged on the greenhouse bench in such a way that there was a distance of four inches between pots. Care was taken in watering that there was no splashing from one plant or pot to another. The results of these trials recorded 68 days after inoculation are given in table 3.

TABLE 3. Results of Inoculation of Sweet Peas and Garden Peas with Non-pathogenic Apple Organisms Resembling *Pseudomonas tumefaciens*

| Culture Number | Sweet Peas | | Garden Peas | |
|------------------------------|-------------------|------------------|-------------------|------------------|
| | Plants Inoculated | Plants Fasciated | Plants Inoculated | Plants Fasciated |
| 597..... | 5 | 1 | 3 | 0 |
| 15..... | 6 | 2 | 2 | 0 |
| 98..... | 4 | 0 | 2 | 0 |
| 123..... | 3 | 1 | 1 | 0 |
| 33..... | 3 | 0 | 2 | 0 |
| 119..... | 2 | 0 | 1 | 0 |
| 43..... | 2 | 0 | 1 | 0 |
| 130..... | 5 | 0 | 2 | 0 |
| 27..... | 3 | 2 | 3 | 0 |
| 162..... | 6 | 0 | 2 | 0 |
| 139..... | 3 | 0 | 2 | 0 |
| 131..... | 3 | 2 | 3 | 0 |
| 133..... | 4 | 2 | 2 | 0 |
| <i>Ps. tumefaciens</i> | 5 * | 0 | 2 * | 0 |
| Checks not inoculated..... | 44 | 9 | 20 | 0 |

* A crown gall on each plant.

The results of these inoculation trials show that fasciation may occur upon sweet peas independently of inoculation with the raspberry strain of the crown-gall pathogen or the non-pathogenic organisms closely resembling

Ps. tumefaciens. The raspberry strain of this species produced typical galls in each case, but no fasciation (Pl. XV A).

At the time that tissue plantings were made from the brown lesions on the root of the first fasciated specimen, dilution plates were also poured from macerated tissue of the basal portion or neck of the abnormal stem growth. The following method was employed in the isolation trials. The portion of the sweet pea plant bearing the fasciation was first washed in tap water to remove adhering soil particles. After drying between clean filter paper, the base or neck of the fasciation was covered with 95 percent alcohol and flamed. The flaming was repeated. Small pieces of the swollen base of the fasciation were removed aseptically to a sterile petri dish; 2 cc. of sterile water were added, and the material finely macerated with a sterile scalpel and allowed to stand one hour. Poured plates were then made in crystal violet bile or potato dextrose agar, using 0.25 cc. of the suspension from the macerated material for each 10 cc. of agar. In some cases a small piece of the basal tissue was crushed in sterile peptone dextrose bouillon. In this case loop dilution plates were made, usually in 18 to 24 hours, after the bouillon had shown slight bacterial growth.

In the first isolation trial, of the 39 poured plates made, 36 remained sterile. In three plates there was a sparse development of small, circular, white, opaque, slightly raised bacterial colonies, about one millimeter in diameter. These colonies were not typical of those of *Ps. tumefaciens*, which developed in the check plates made from crown gall on tomato plants. In subsequent trials at frequent intervals, other isolations were made involving over 450 plates and 33 different specimens of fasciated sweet pea plants. For the most part, the bacterial colonies which came up on the plates were not typical of those of the crown-gall organism. However, in some of the isolation trials a few colonies were obtained which bore a very close resemblance in streak and plate cultures to *Ps. tumefaciens*. These cultures were employed in inoculation trials on young plants of sweet pea, sugar beet, and tomato. In the case of the sweet pea, the seeds were first disinfected in 1-500 mercuric chlorid solution, rinsed in sterile distilled water, and germinated between filter paper in sterile petri dishes or directly in soil steamed for 6 hours at 30 pounds pressure. The seedling, with the plumule not over one and one-half inches in length, was inoculated at a point just above the point of attachment to the cotyledons. After inoculation, the seedlings were planted in pots of steamed soil so that the point of inoculation was buried to a depth of one inch. The sugar beet plants also grown in steamed soil were inoculated at the crown of the plant, usually about one-fourth inch below the surface of the soil. The young tomato plants, usually about six inches in height, were inoculated at the tip of the growing stem.

The results of the inoculation trials with 12 organisms isolated from fasciations on the sweet pea are summarized in table 4.

Table 4 shows that of the 339 sweet pea plants inoculated with the organisms resembling *Ps. tumefaciens* recovered in poured plates from fasciations, only 54 or 15.9 percent developed the abnormality. In the checks, 4 out of 59 or 6.7 percent of the uninoculated plants developed

TABLE 4. *Results of Inoculations with Bacterial Organisms Isolated from Sweet Pea Fasciation*

| Organism | Plants Inoculated and Results | | | | | |
|------------------------------------|-------------------------------|---------------|------------|------------|------------|------------|
| | Sweet Pea | | Tomato | | Sugar Beet | |
| | No. Plants | No. Fasciated | No. Plants | No. Galled | No. Plants | No. Galled |
| Bacteria from fasciation | 339 | 54 | 36 | 0 | 30 | 0 |
| <i>Ps. tumefaciens</i> | 49 | 2 | 12 | 12 | 12 | 12 |
| Checks | 59 | 4 | 12 | 0 | 12 | 0 |

fasciation. Two of the 49 plants inoculated with the raspberry strain of *Ps. tumefaciens* showed fasciation. In this case, the fasciated plants occurred in the same pot during one experiment. No galls resulted from this inoculation (8 plants) but they occurred in a high percentage of cases in all previous inoculation trials with the crown-gall organism. It will be noted, also, that in no case did the bacterial organism from sweet pea fasciation induce galls on either sugar beet or tomato.

These results indicate that factors other than the bacteria isolated from the fasciation, as well as the strain of *Ps. tumefaciens* employed, may also be responsible for the development of this abnormality on the sweet pea.

RELATION OF INSECT INFESTATION TO FASCIATION

At various times during the course of these experiments, examinations of fasciated sweet pea plants in commercial beds showed the presence of large numbers of larvae of the mealy bug (*Pseudococcus* sp.).¹ Although this insect has not been proved to cause such abnormalities, it appeared that the larvae might be associated causally with fasciation. A heavy infestation, with mites, of *Cyclamen indicum* growing in a nearby bed also suggested the possibility of their association with the abnormalities upon the sweet pea.

Experiments, therefore, were carried out in which the larvae of the mealy bug and mites from the *Cyclamen* were allowed to feed upon the underground portions of young sweet pea plants. The sweet pea plants used in these trials were grown from disinfected seed in steamed soil. Portions of fasciations bearing numerous mealy bug larvae and leaves of the *Cyclamen* heavily infested with mites were buried about the crowns of

¹ The writers are indebted to Dr. Harold Morrison, Bureau of Entomology, U. S. Department of Agriculture, for the identification of the mealy bug larvae.

young sweet pea plants when three to four inches in height. Each pot was banded with tanglefoot and placed upon an inverted ten-inch pot to prevent escape of the larvae and mites and the access of wingless insects. Other plants punctured with a sterile needle and those inoculated with *Ps. tumefaciens* were held as checks. The plants were inoculated or infested February 15 and the final readings made May 24. These data are summarized in table 5.

TABLE 5. *Results of Infesting Sweet Pea Plants with Mealy Bug Larvae and Cyclamen Mites, and Inoculation with Pseudomonas tumefaciens*

| Treatment of Plants | Number of Plants | | | Percent Fasciated |
|-------------------------------------|------------------|-----|-----------|-------------------|
| | Before Treatment | Dug | Fasciated | |
| None (check)..... | 89 | 71 | 8 | 11.2 |
| Mealy bug larvae..... | 50 | 39 | 5 | 17.2 |
| Cyclamen mites..... | 17 | 4 | 0 | 0 |
| <i>Ps. tumefaciens</i> (check)..... | 15 | 8 * | 0 | 0 |

* No fasciations; galls on all plants.

The data presented in table 5 are inconclusive with regard to the cause of fasciation on the sweet pea. The fact that the untreated check plants show almost as high a percentage of fasciations as those infested with mealy bug larvae indicates that these insects alone are not the cause of the abnormality. The cyclamen mites caused the death of 13 of the 17 plants infested and the remaining four were not fasciated. None of the plants inoculated with *Pseudomonas tumefaciens* developed fasciations although all of them showed typical crown galls.

A second lot of sweet pea plants was infested with the larvae of the mealy bug, *Pseudococcus* sp., in an effort to determine if there was any correlation between infestation and the development of fasciations. For this experiment, seeds of four varieties, Ball Rose, Vulcan, Lavender King, and Vaughan's Mixture, were immersed for ten minutes in a 1-500 solution of mercuric chlorid and germinated between moist filter paper in sterile petri dishes. When the plumules of the germinating seeds were one-fourth to one-half inch in length, five larvae of the mealy bug with a mass of eggs were placed upon the root of each plant. The infested plants were then set in steamed soil in disinfected pots. A small celluloid cylinder was set in the soil, the young infested plant set within it, and soil to a depth of one inch added. The cylinder was then plugged with cotton to prevent the escape of the mealy bugs and to keep out other insects. Other plants were infested in a like manner, but not placed within cylinders. Plants were left untreated and also inoculated with *Pseudomonas tumefaciens* as checks. The results of this experiment taken 60 days after planting are given in table 6.

The data from this experiment are in accord with those given in table 4 and show that there is no causal relation between mealy bug infestation of sweet pea plants and the development of fasciation. While the infested

TABLE 6. *Results of Infesting Sweet Pea Plants with Larvae of the Mealy Bug, Pseudococcus sp.*

| Treatment | Number Plants | Number Fasciated |
|--|---------------|------------------|
| None (check)..... | 82 | 5 |
| Plants in cylinders. Infested..... | 37 | 6 |
| Plants not in cylinders. Infested..... | 33 | 1 |
| Plants in cylinders. Not inoculated..... | 16 | 0 |
| Plants in cylinders inoculated with <i>Ps. tumefaciens</i> | 7 * | 0 |

* All plants with galls.

plants show some fasciation, the same condition is also found on the untreated check plants. In both cases the percentage of fasciated plants is too small to be significant. No fasciation resulted from inoculation with the raspberry isolation of the crown gall organism.

SEED TRANSMISSION

An attempt was made to determine if the fasciated condition might be transmitted through the seed. For this experiment seed was taken from sweet pea plants, with and without fasciations, grown in steamed soil.

The seed, after disinfection in 1-500 mercuric chlorid solution for 10 minutes, was planted in pots of steamed soil. The pots were then sunk in steamed soil in disinfected flats. Three flats containing pots of seed from fasciated and normal plants were then placed in cages with tight wooden bottoms and covered with closely woven cloth to exclude insects. The cages were placed upon inverted five-inch pots in a pan of water and the surface of the water was covered with kerosene. As checks, pots of the same lots of seed similarly treated were sunk in flats of steamed soil, but left outside the insect-proof cages. After the plants were 53 days old readings on the occurrence of fasciations were made.

TABLE 7. *Relation of Seed Transmission to Fasciation in Sweet Peas*

| Treatment | Source of Seed | No. Plants | No. Fasciated |
|-----------------------|------------------|------------|---------------|
| Plants caged..... | Fasciated plants | 31 | 0 |
| | Normal plants | 28 | 0 |
| Plants not caged..... | Fasciated plants | 78 | 4 |
| | Normal plants | 60 | 2 |

As shown in table 7 there were 31 plants from the seed of fasciated and 28 plants from seed of normal plants grown under cloth cages to exclude

insects. None of the 59 plants showed fasciations. Similarly, in the open there were 78 plants from seed of fasciated and 60 plants from seed taken from normal plants. Four of the 78 plants, and two of the 60 plants, showed fasciations. The plants grown in the open displayed more vigorous growth than those within the cages. These results indicate that fasciation in the sweet pea plant is not transmitted through the seed, but is more probably due to environmental or nutritional conditions.

THE RELATION OF ENVIRONMENTAL CONDITIONS TO FASCIATION

In commercial practice, sweet pea seed is frequently first planted in three-inch pots, three seeds in each pot. When the young plants are about one month old they are set in the beds. It has been noted repeatedly that the young plants, when 10 to 12 inches in height at the time of transplanting into greenhouse ground beds, show a high percentage of fasciation. After transplanting, the abnormality continues to develop with the increased growth of the plant.

It appeared that the abundant development of roots and subsequent "root-binding" in the small pots may have been responsible for the development of the fasciated plants. In previous trials sweet pea plants from disinfected seed were grown in flats of steamed soil and transplanted to the greenhouse bed, and into small pots. In the latter case, four seedlings were planted in each three-inch pot and the pot sunk to one-third its depth in clean river sand. The second lot of seedlings transplanted to the small pots were about three weeks older than those planted in the greenhouse bed. In these trials 265 plants were grown in the greenhouse bed and 346 in three-inch pots. Since under commercial conditions they showed relatively high percentages of fasciated plants, the following varieties were used: Snow-storm Improved, Vulcan, Lavender King, and Zvolanek's Rose. The plants in the greenhouse beds were allowed to grow until they were blossoming in abundance. No fasciations developed in any of the varieties. When the seedlings of the plants grown in three-inch pots were about one inch in height, those in approximately half of the pots of each variety were wounded by puncturing the base of the stem with a sterile needle. The plants in the three-inch pots were not so vigorous as those in the bed, but attained a height of about 15 inches at the end of 52 days. At this time the experiment was terminated because of the dying of some of the plants. The number of plants of each variety and treatment with the number fasciated are shown in table 8.

From the data in table 8 it is seen that no fasciations developed on the plants grown in steamed soil in the greenhouse bed, while a high percentage of those in small pots showed the abnormality. It is interesting to note also that wounding of the young plants did not materially increase the percentage of fasciation. The data from the two plantings also showed that fasciations can develop in the absence of artificial insect infestation or

bacterial infection. The results of these trials also show that fasciation developed only on those plants in the three-inch pots where there was overcrowding of the roots.

TABLE 8. *Relation of Environment and Mechanical Injury to the Development of Fasciations in the Sweet Pea*

| Variety | Plants Grown | | | | | |
|-------------------------|-------------------|---------------|--------------------|---------------|-------------|---------------|
| | In Greenhouse Bed | | In Three-inch Pots | | | |
| | | | Wounded | | Not Wounded | |
| | No. Plants | No. Fasciated | No. Plants | No. Fasciated | No. Plants | No. Fasciated |
| Zvolanek's Rose..... | 95 | 0 | 19 | 2 | 33 | 8 |
| Snowstorm Improved..... | 22 | 0 | 27 | 9 | 40 | 13 |
| Lavender King..... | 62 | 0 | 60 | 24 | 75 | 4 |
| Vulcan..... | 64 | 0 | 25 | 2 | 67 | 12 |

A further experiment was carried out, in which disinfected seed was planted at the same time in a bed of steam sterilized soil and in three-inch pots of similarly treated soil alongside the bed. In this way, both lots of plants were placed under identical conditions with the exception of the crowding of the roots on those plants in the small pots. The seeds were planted June 24 and the final readings made September 4, when the plants were in blossom. The data, presented in table 9, show that the highest percentage of fasciation occurred on those plants grown in small pots where crowding of the roots took place. In the case of the variety Zvolanek's Rose, it is interesting to note that whereas 27.56 percent of the plants in the pots showed fasciation (Pl. XV B), none of the plants grown alongside in the bed under similar conditions were so affected. In a like manner, the plants of the variety Snowstorm, grown in the bed, showed 1.36 percent fasciation, while of those grown in small pots, 21.8 percent were fasciated. In the variety Lavender King, the difference in the percentage of fasciation in the plants grown in the bed and in pots is not so great as in the two varieties previously mentioned. In this variety, the fact that the plants in the pots made a very poor growth probably accounted for the small percentage of fasciation. In the variety Lavender King, however, the percentage of fasciation on the plants in pots (11.2) was at least twice that on the plants in the bed. Disregarding the individual varietal reaction, it is seen that the average percentages of fasciation in the plants grown in the beds and in pots is 1.87 and 18.63, respectively.

The results of this experiment show that fasciation of sweet peas may be induced by unfavorable environmental conditions in the absence of crown-gall infection.

TABLE 9. *The Effect of Root Crowding Upon the Development of Fasciation in the Sweet Pea*

| Variety | Planted in Bed | | | Planted in 3-inch Pots | | |
|------------------------|----------------|---------------|-------------------|------------------------|---------------|-------------------|
| | No. Plants | No. Fasciated | Percent Fasciated | No. Plants | No. Fasciated | Percent Fasciated |
| Zvolanek's Rose..... | 84 | 0 | 0 | 156 | 43 | 27.56 |
| Snowstorm..... | 73 | 1 | 1.36 | 133 | 29 | 21.80 |
| Lavender King..... | 94 | 5 | 5.31 | 194 | 23 | 11.8 |
| Vulcan..... | 122 | 1 | 0.81 | 177 | 27 | 15.2 |
| Total and average..... | 373 | 7 | 1.87 | 660 | 102 | 18.63 |

SUMMARY

Fasciation of sweet peas has been observed in the greenhouse on the following varieties: Zvolanek's Rose, Snowstorm Improved, Yarrows, Mrs. Cuthbertson, Vulcan, Lavender King, Spencer's Mixed, Louise Gude, Eldorado, and Ball Rose.

Isolations made from a brown lesion on the stem below the fasciation on certain plants yielded mostly *Fusaria*. Isolations from the neck or base of the fasciation in a few cases yielded bacteria resembling the crown-gall organism in plate and slant cultures.

Plants grown from disinfected seed in sterilized soil were inoculated with cultures of the various fungi and bacteria obtained from fasciated plants as well as with *Rhizoctonia Solani*, *Pseudomonas tumefaciens* (raspberry strain), and 12 non-pathogenic bacteria closely resembling *Ps. tumefaciens*. Fasciation developed upon certain of the inoculated plants and also upon the uninoculated, unwounded check plants.

The bacterial organisms closely resembling *Ps. tumefaciens* in culture, isolated from fasciated sweet peas, failed to produce crown gall when inoculated into young sugar beet and tomato plants.

Fasciation developed on plants grown from disinfected seed in sterilized soil when they were infested with the larvae of the mealy bug. Plants grown in a similar manner but infested with the cyclamen mite showed no fasciation. The non-infested check plants grown under similar conditions also showed fasciation.

No evidence of seed transmission of fasciation was obtained.

In sweet peas grown from disinfected seed in steamed soil in a greenhouse bench an average of 1.8 percent of the plants developed fasciations. Other plants grown alongside but in three-inch pots where the roots were crowded showed an average of 18.6 percent of fasciated plants.

Fasciation in sweet peas apparently may develop in the absence of the crown-gall organism.

These studies have been carried out at the Iowa State College in con-

nection with the crown gall project in which the U. S. Department of Agriculture, the Crop Protection Institute of the National Research Council, the University of Wisconsin, and the Iowa State College are coöperating. The writers wish to acknowledge their indebtedness to Dr. I. E. Melhus for helpful criticisms and suggestions during the course of these investigations and in the preparation of the manuscript.

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DESCRIPTION OF PLATES

PLATE XIV

A. Fasciated sweet pea plants of the variety Mrs. Culbertson, showing a brown lesion on the lower portion of the stem. The first isolation trials were made from the lesion and fasciation on this specimen.

B. Fasciated plants, variety Vaughan's Mixture, grown in a greenhouse bed in which a high percentage of the previous crop showed the abnormality.

PLATE XV

A. Crown gall on Zvolanek's Rose sweet pea induced by inoculation with *Pseudomonas tumefaciens* isolated from red raspberry.

B. Check plant of the same variety, grown from disinfected seed in steam sterilized oil. Plant not wounded nor inoculated.



MUNCIE AND PATEL: FASCIATION



MUNCIE AND PATEL: FASCIATION

GENERA OF NORTH AMERICAN FABACEAE

VII. *ASTRAGALUS* AND RELATED GENERA (CONTINUED)

P. A. RYDBERG

(Received for publication October 18, 1929)

21. *Hamosa* Medic. Vorl. Churph. Phys. Ges. 2: 373. 1787

Annual or perennial herbs. The leaves are alternate, odd-pinnate, with several or many leaflets. The flowers are perfect, in axillary racemes. The calyx-tube is campanulate, the teeth subulate. The corolla is purple or white, rarely ochroleucous. The banner is broadly obovate, subsessile. The wings are clawed, the blade obliquely oblanceolate or lunate, with a reflexed auricle, free. The keel-petals are adnate, clawed, the blades broadly lunate, in two American species produced into a small beak. The stamens are diadelphous (9 and 1), the sheath is straight, the free upper part of the filaments curved upwards. The ovary is many-ovuled, the style curved, the stigma minute, terminal. The pod is elongate, sessile, linear or oblong, or rarely lance-oblong in outline, usually somewhat compressed, rarely terete, coriaceous, or rarely membranous, not inflated, completely (or in one species incompletely) 2-celled, the septum meeting the upper suture. The seeds are obliquely reniform.

ILLUSTRATION: Plate XVI *U. Hamosa Nuttalliana* (DC.) Rydb., $\times 2/3$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil, $\times 2$; 7. pod; 8. pod in longitudinal section, the septum partly removed to show the seeds, $\times 1$; 9. pod in cross-section; 10. seed, $\times 2$.

SYNONYM: *Hamaria* Fourr, Ann. Soc. Linn. Lyon, II. 16: 364. 1868.

Fourreau changed the name to *Hamaria*, a noun meaning a bearer or user of hooks, as *hamosa* is an adjective, meaning full of or abundant in hooks. Medicus, who established the genus on *Astragalus hamosus* L., used the feminine adjective form of the specific name as generic name. He also used several other specific adjectives in a similar way for his genera. The worst of these is perhaps *Contortuplicata*, another genus of this group. In the type species, *Astragalus hamosus*, the pod is much elongate, semi-woody or firmly leathery, completely 2-celled, and strongly curved. The main difference between *Hamosa* and *Astragalus*, in a restricted sense, is the elongate, spreading, or reflexed pod of the former. Only in a few of the species (in none of the American ones), the pod is as much curved as in the type; in many of them the pod is straight. In the North American species the texture of the valves is less thick and less firm, in some sub-membranous. In one species, *Hamosa imperfecta*, the septum does not meet the upper suture, but in every other respect the structure of the pod and the habit of the plant agrees perfectly with the other species of the section LEPTOCARPAE. In two species, *H. nothoxys* and *H. acutirostris*,

the keel-petals are produced into a distinct beak. These two species sometimes have been transferred to *Oxytropis*, but outside of this feature, they have nothing in common with that genus.

The genus is found in both the Old World and in North America. The species in the latter number 59, which can be divided into 8 sections. The Old World species are rather few and represent other sections of their own.

22. *Cystium* Steven, Bull. Soc. Nat. Mosc. 29²: 147. 1856

Leafy-stemmed annual or perennial herbs. The leaves are odd-pinnate, with broad leaflets and nearly free stipules. The flowers are usually borne in long-peduncled axillary racemes. The calyx-tube is campanulate, the teeth subulate. The corollas are white or purplish, middle sized. The banner is obovate, sub-sessile, often retuse, slightly arcuate. The wings are clawed, the blade oblanceolate, with a large basal auricle. The keel-petals are clawed, the blade broadly lunate, with a small auricle. The stamens are diadelphous (9 and 1), the sheath nearly straight, broad at the base, the free portion of the stamens curved upward. The ovary is sessile, many-ovuled, straight, the style curved upwards above the middle, glabrous, the stigma minute. The pod is sessile, papery or parchment-like, much inflated, from orbicular to ovoid, the tip usually up-curved, perfectly 2-celled, the septum from the lower suture reaching the upper. Seeds obliquely round-reniform.

ILLUSTRATION: Plate XVI V. *Cystium diphsyun* (A. Gray) Rydb. $\times 2/3$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil; 7. pod, dorsal view; 8. pod, side-view; 9. pod, in longitudinal section, with a part of the septum removed in order to show the seeds; 10. pod, in cross-section, $\times 1$; 11. seed, $\times 2$.

SYNONYM: *Glaux* Medic. Vorl. Churph. Phys. Ges. 2: 374. 1787. Not *Glaux* L. 1753.

This genus was also established by Medicus under the name *Glaux*. As the name was preoccupied by *Glaux* L., we must adopt the later name *Cystium* Steven. The type of both was *Astragalus Cicer* L. The pod is of the form and texture of that of *Phaca*, usually sessile but sometimes short-stipitate, strongly inflated and papery, but completely 2-celled. It corresponds to Gray's section DIPHYSI of *Astragalus*.

It comprises 32 North American species native of the western part of the continent. It is also represented in Europe and northern Asia.

23. *Onix* Medic. Vorl. Churph. Phys. Ges. 2: 374. 1787

Leafy-stemmed perennials. The leaves are odd-pinnate, with many leaflets and distinct stipules. The flowers are borne in peduncled axillary racemes. The calyx-tube is campanulate. The corolla is white or yellowish. The banner is obovate, subsessile, retuse. The wings are clawed, the blade lunate-oblanceolate, with a large basal auricle. The keel-petals are shorter and broader, the blade almost semi-circular. The stamens are diadelphous (9 and 1), the sheath straight, the free portion of the filaments abruptly bent upwards. The ovary is stipitate, rather few-ovuled, the style bent

upwards, the stigma minute. The pod is membranous, inflated, triangular or cordate in cross-section, completely 2-celled, in age splitting through the septum. The seeds are obliquely reniform.

ILLUSTRATION: Plate XVI **W.** *Onix galegiformis* (L.) Med., $\times 2/3$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil; 7. pod, side-view; 8. pod, dorsal view; 9. pod, split in two, in the lower half its half of the sceptor is shown in position, in the upper half the sceptor is removed, showing the seeds; 10. pod, in cross-section, $\times 1$; 11. pod of *Onix Mulfordae* (M. E. Jones) Rydb., in side-view; 12. pod, in dorsal view, $\times 1$; 13. pod, in cross-section; 14. seed, $\times 2$.

This genus was also proposed by Medicus with *A. galegiformis* L. as the type. The pod resembles that of the preceding genus, being inflated and completely 2-celled, but it is long-stipitate, and at maturity splits in two.

In western North America the genus is represented by but one species, *O. Mulfordae* (M. E. Jones) Rydb.; there are a few in Eurasia.

24. *Geoprumnon* Rydb.; Small, Fl. SE. U.S. 615, 1332. 1903

Low perennials, with cespitose rootstocks. The stems are mostly decumbent or spreading. The leaves are odd-pinnate, with stipules but without stipels, the leaflets petioluled. The flowers are borne in axillary dense racemes. The calyx is deeply campanulate, the tube longer than the 5 teeth. The corolla is purple or whitish. The banner is oblanceolate or narrowly obovate, mostly retuse at the apex, cuneate at the base, without a claw, moderately arched at the middle, with spreading margins. The wings have an oblong blade about equalling the claw and a basal reflexed auricle. The keel-petals are shorter, the blade broadly lunate, obtuse at the apex. The stamens are diadelphous (9 and 1), the sheath almost straight. The ovary is sessile, many-ovuled, the style bent above, glabrous, the stigmas capitate. The pod is ellipsoid to sub-globose, completely 2-celled by the intrusion of the lower suture, fleshy, becoming spongy in age, tardily dehiscent. The seeds are round-reniform, oblique.

ILLUSTRATION: Plate XVI **X.** *Geoprumnon crassicaipum* (Nutt.) Rydb., $\times 2/3$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil; 7. pod; 8. pod, in cross-section, $\times 1$; 9. seed, $\times 2$.

This genus was established in 1903, with *Astragalus crassicaipus* as the type. It consists of 7 low, spreading species from the prairie and plain regions of the Mississippi basin. The genus is represented nowhere else. It is distinguished from all the other genera by the fleshy pods, which were used as a food by the Indians and the early settlers.

25. *Hesperastragalus* Heller, Muhlenbergia 2: 86. 1905

Slender annuals, or rarely (in the Mexican species) perennials. The leaves are alternate, odd-pinnate, the stipules distinct and almost free from the petioles. The flowers are perfect, borne in dense racemes or spikes, with minute bracts and very short pedicels. The calyx-tube is hemispheric or short-campanulate, the teeth nearly equal, mostly subulate. The corolla is small. The banner is oblanceolate, clawless, usually retuse

or notched at the apex. The wings are shorter, the blade equaling the claw, mostly oblong, scarcely falcate, with a large basal auricle. The keel-petals are shorter, the blade lunate to semi-orbicular, often shorter than the claw and with a small auricle. The stamens are diadelphous (9 and 1), the sheath nearly straight, the free portion of the filaments abruptly bent upwards. The pistil is sessile or nearly so, the ovary short, with 1-3 ovules in each cell, style glabrous, the stigma capitate. The pod is completely 2-celled, in fruit reflexed, short, coriaceous, almost didymous, usually with 1-3 seeds in each cell, the seeds almost filling it, the valves more or less cross-veined or cross-ribbed.

ILLUSTRATION: Plate XVII Y. *Hesperastragalus didymocarpus* (H. & A.) A. Heller, $\times 2/3$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil; 7. pod, in side-view; 8. pod, in dorsal view; 9. one of the semi-carpels seen from the inner face; 10. pod, in cross-section, $\times 3$; 11. seed, $\times 2$.

The genus was described in 1905 by A. Heller, *Astragalus didymocarpus* H. & A. serving as the type. It differs from *Astragalus* in the small 2-celled, reflexed, more or less didymous, few-seeded, indehiscent, ribbed fruit. It comprises the sections REFLEXI and DIDYMOCARPI of Dr. Gray and MICROLOBIUM of Nuttall. It consists of 13 species from western and southwestern United States and Mexico.

26. *Hesperonix* Rydb. N. Am. Fl. 24: 438. 1929

Leafy-stemmed perennials. The leaves are odd-pinnate, with many leaflets and distinct stipules. The flowers are born in peduncled axillary racemes. The calyx-tube is campanulate, the teeth subulate. The corolla is mostly white or ochroleucous. The banner is obovate, sessile, mostly retuse. The wings are clawed, the blades obliquely oblanceolate, with a basal auricle. The keel-petals have shorter and broader lunate blades. The stamens are diadelphous (9 and 1), the sheath is straight, the free portion of the filaments curved upwards. The ovary is stipitate, many-ovuled, the style glabrous, curved upwards, the stigma minute, terminal. The pod is leathery or coriaceous, inflated, stipitate, completely 2-celled, the septum formed by the lower suture extending to the upper. The seeds are obliquely reniform.

ILLUSTRATION: Plate XVII Z. *Hesperonix Bolanderi* (A. Gray) Rydb., $\times 2/3$; 1. calyx; 2. keel-petal; 3. wing; 4. banner, $\times 1$; 5. stamens; 6. pistil, $\times 2$; 7. pod, in side view; 8. pod, in ventral view; 9. pod, in longitudinal section, showing the septum, which does not quite reach the apex; 10. pod, in cross-section, $\times 1$; 11. seed, $\times 2$.

The genus was established in the North American Flora in 1929, and based on 6 species from the Pacific Slope of North America and 1 from the Rocky Mountain region. It is not represented in the Old World. At first, the author was inclined to include them in *Onix*, as the pod is inflated, 2-celled and stipitate, but the valves are of a firmer structure and the pod does not split into 2 halves as in *Onix*.

There are also two of the European segregates of *Astragalus* represented

in North America by introduced species. As they do not really belong to our flora, a complete diagnosis is not given here. As an illustration of the first had been prepared, it is here included. It is sufficient to point out the main characters by which these segregates are distinguished from *Astragalus*.

Hedyphylla Steven, Bull. Soc. Nat. Mosc. 29²: 142. 1856

This genus was established in 1856 and based on two species *Astragalus glycyphyllus* and *A. glycyphylloides*, from Europe and northern Asia. If there are any more species to be included in it I do not know. The genus is characterized by the elongate, turgid, somewhat inflated pod. In the young state the cavity is filled with a spongy tissue which in age becomes fibrous.

ILLUSTRATION: Plate XVII A. *Hedyphylla glycyphylla* (L.) Rydb., $\times 2/3$; 1. calyx, $\times 2/3$; 2. banner; 3. wing; 4. keel-petal, $\times 1$; 5. stamens; 6. pistil, $\times 2$; 7. pod, in side-view; 8. pod, in longitudinal section, a part of the septum removed to show the seeds, $\times 2/3$; pod, in cross-section; 10. seed, $\times 2$.

Steven had recognized that *Astragalus* was not a natural genus and in 1832 he tentatively split it up into several groups. These groups he gave substantive names; but, as he did not connect these names with binomials, only indicating which species of *Astragalus* should be included in them, these names cannot be regarded as genera, but rather as sections. One of these was *Glycyphylla*. (See Bull. Soc. Nat. Mosc. 4: 266. 1832.) In 1856, he raised these sections to generic ranks, adding also some new ones. He changed the name to the section mentioned above to *Hedyphylla*, probably because there was an older *Glycyphylla* Raf. (1819). Neither did he conserve the Linnean specific name *glycyphylus* under *Astragalus*, but called his type *Hedyphylla vulgaris*. *H. glycyphylla* has been collected as a rare adventive around a few Atlantic seaports.

Contortuplicata Medic. Vorl. Churph. Phys. Ges. 2: 378. 1787

This was based on *Astragalus contortuplicatus* L., which has been collected at Westport, Massachusetts. The name used by Medicus was *Contortuplicata astragaloides*. I regret very much in having been forced to make such a combination as *Contortuplicata contortuplicata* (L.) Rydb.

The genus is characterized by its remarkable pod, which resembles that of *Hamosa* in being elongate, 2-celled and curved, but it is of a more delicate texture and splits very early lengthwise into two closed false carpels. The general habit is quite unlike that of any of the other *Astragalus* segregates. Evidently the genus is monotypic, a native of the Old World.

4. OXYTROPOID GENERA, usually with a partial septum formed by the upper suture; keel-petals beaked.

27. *Oxytropis* DC. Astrag. 24: 66. 1802

Perennial herbs, with a caudex or rootstock, usually sub-accaulescent, with short branches or rarely (in the North American species only in some of the section DEFLEXAE) with better developed leafy stems. The leaves are borne mostly near the base, odd-pinnate with several or many leaflets, sometimes arranged verticillate on the rachis. The flowers are mostly in dense spikes on elongate peduncles. The calyx-tube is cylindric, the teeth subulate. The corolla is mostly purple or ochroleucous, rarely white. The banner is obovate, often with a broad claw. The wings are usually long-clawed, the blade broad, oblique, sometimes 2-lobed, with a large incurved basal auricle. The keel-petals are similar, but shorter and produced at the apex into a distinct beak. The stamens are diadelphous (9 and 1), the sheath straight, and the short free portion of the filaments up-curved. The ovary is usually sessile, the style glabrous, slightly curved near the apex, the stigma minute. The pod is mostly sessile, from membranous to leathery in texture, elliptic, ovoid, or lance-ovoid in outline, turgid or somewhat inflated, many-seeded, the upper suture usually more or less inflexed, the lower rounded, never intruding. The seeds are obliquely reniform, with a deep-seeded hilum.

ILLUSTRATION: Plate XVII I. *Oxytropis Lambertii* Pursh, $\times 2/3$; 1. calyx; 2. banner; 3. wing; 4. keel-petal; 5. stamens; 6. pistil; 7. pod, $\times 1$; 8. pod, in cross-section; 9. seed, $\times 2$.

SYNONYMS:

Aragallus Necker, Elem. 3: 12, in part. 1790.

Spiesia Necker, Elem. 3: 13 (hyponym). 1790.

As the type must be regarded *Oxytropis montana* (L.) DC., based on *Astragalus montanus* L. It contains about 70 North American species and even more in Eurasia.

The genus was established in 1802, by De Candolle (Astrag. 3 and 6, 1802), and distinguished from *Astragalus* by two characters: the keel-petals ending in a distinct beak and the upper suture (not the lower) inflexed and more or less transformed into a partial septum. Many new species having been added to the two genera since De Candolle's time and better knowledge having been gained on others, these characters no longer hold absolutely. Two species of *Ilamosa*, a segregate from *Astragalus*, possess a beak, but the lower suture forms a complete septum. In several species of *Oxytropis*, the upper suture may be slightly inflexed but no septum is formed; in a few the upper suture is not inflexed at all and the pod is that of *Phaca* in all respects. If these species (all Old World species) are removed from *Oxytropis*, the genus is a very natural one, both as to the structure of the pod and as to the general habit.

If the limitation of the genus can be settled easily, it is not so with the name the genus should bear. De Candolle had overlooked that there were two older names that might be applied to his genus, *Aragallus* and *Spiesia* of Necker. I say that he had overlooked these because he does not mention them in his list of older names applying to *Astragalus* and its relatives. Neither did he mention any of Medicus' segregates. He might have dis-

regarded them as being not properly established, or imperfectly known, or not based on good characters. There is no doubt what Medicus' genera represent, but none of them applies to *Oxytropis*. Necker's genera, however, apparently, refer to this genus at least in part.

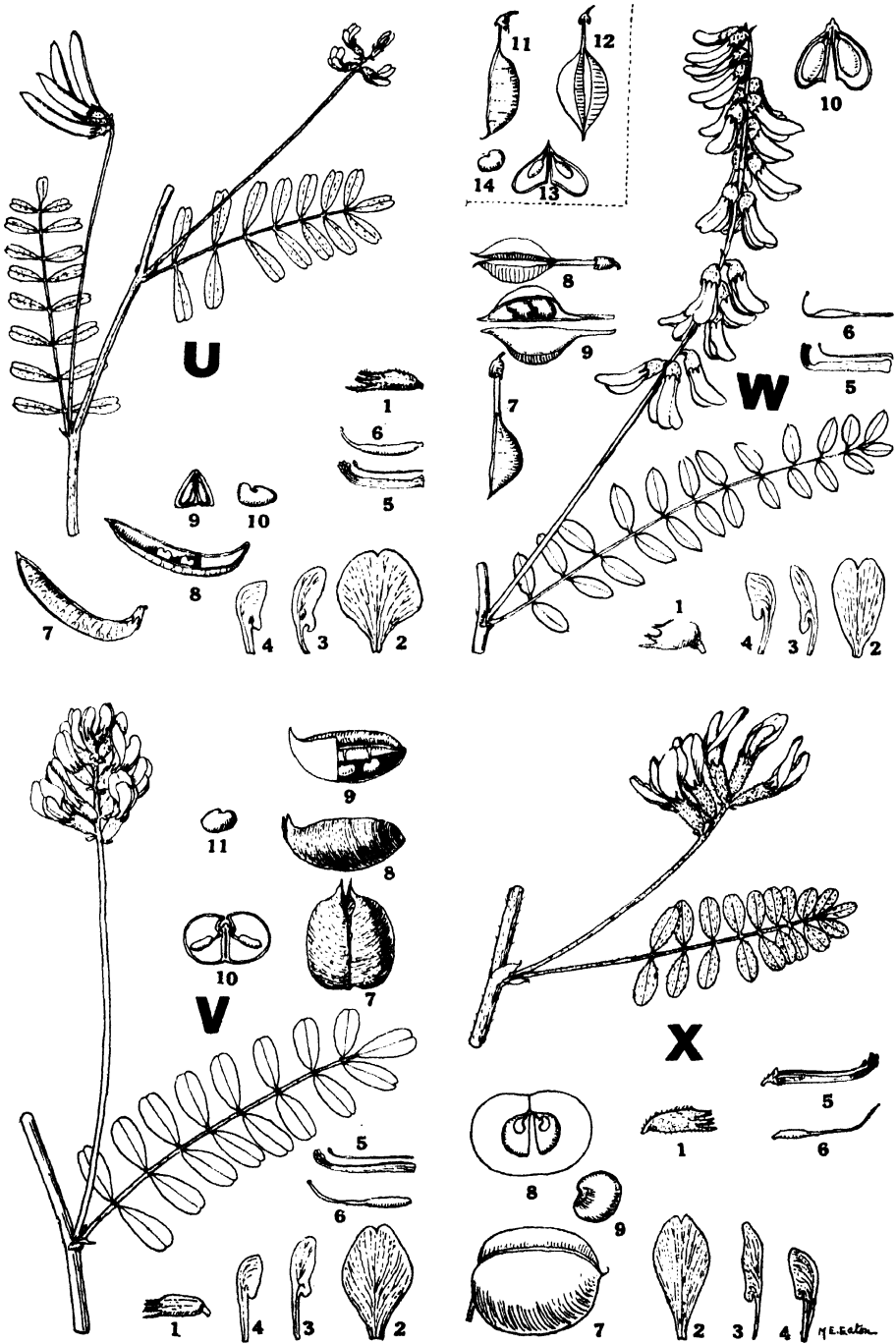
To one that follows the International rules of nomenclature, there is no trouble in selecting the name to be used, as *Oxytropis* is a *nomen conservandum* against *Spiesia* and by implication also against *Aragallus*. One that follows the American Code has more trouble. Otto Kuntze in 1891 (see Rev. Gen. 205) adopted Necker's *Spiesia*, claiming that it was based on *Phaca muricata* and *P. lanata* Pallas. Necker does not say so. He only says "Quaed. Phac. Pallas," i.e., some species of *Phaca* of Pallas, why not *P. prostrata* and *P. microphylla* also? No definite species was cited and no characters are given that separate the genus from *Phaca*. It is true that *Phaca muricata* might be one of the species, as the legume is described as muricate, but that fact does not make it the type of the genus. The pod is described as being 1-celled in *Spiesia* and sub 2-celled in *Phaca*. In fact, in the original species of *Phaca* L. there is no trace of a partition, and in *Phaca muricata* the pod is partly 2-celled by a partial septum formed by the upper suture. If *P. muricata* Pallas is the type, the genus is wrongly characterized, separated from *Phaca* by characters not existing. At most, *Spiesia* can be said to be a genus, poorly characterized, based on non-existing characters, with vague application and without a definitely designed type. Should we accept such a genus?

The case of *Aragallus* is somewhat different. It was adopted by Greene in 1897. (See Pittonia 3: 208-212.) Kuntze criticised Greene for doing this, but only on the ground that it had page priority over *Spiesia*. This was one reason, but another was that Greene was inclined to keep the two genera apart and did not transfer to *Aragallus* any of the species of Pallas which might be included in the original *Spiesia*. Dr. Greene states: "De Candolle, it is true, includes them all [Pallas species] in *Oxytropis*, as having the habit of the genus, and one of the characters; but he places them last in the series, on account of their one-celled pods." This later statement is not correct. De Candolle used in his descriptions under the different species of *Oxytropis* referred to above the expressions "not altogether 2-celled or half 2-celled," indicating that the pod has a partial septum. Greene made the mistake in stating that De Candolle placed them in a section that had a 1-celled pod, while the latter placed them in a section with verticillate leaflets. If that is the real character of *Spiesia*, *Astragalus verticillaris*, which both Necker and Greene regarded as belonging to *Aragallus*, should belong to *Spiesia*, and the diagnostic character of the latter genus was not given in print by Necker.

Aragallus has lately been discarded in America on the ground that it was a "hyponym." This is not true. *Aragallus* has a diagnosis covering half a page. In it there is no further reference to a type, except the expressions:

"Quaed. Astrag. Linn.," which means some species of *Astragalus* L. If that was all, we could state that no type or any other species was assigned, but just above the diagnosis, under the genus *Astragalus*, we read: "Species haec. proles 34, distinctos, habit. Ceterae, que in system. veget. ed. XIV. Linn. recensentur, ad speciem sequentem pertinent." In other words *Astragalus* had according to Necker 34 species mentioned in the 14th [Murray's] edition of *Systema Vegetabilium*. The rest in that work belongs to the following genus, *i.e.* *Aragallus*. Under *Aragallus* is also stated that it contains 12 species. The first two divisions of the genus *Astragalus* in the work referred to has 33 species [not, as Necker states, 34, the only error], the third division has 12 species, characterized as scapose, without leafy stems, the only character of any importance that Necker assigned to *Aragallus* as distinguishing it from *Astragalus*. There is, therefore, no doubt what constituted Necker's concept of the genus *Aragallus*, and *Aragallus* is by no means a hyponym.

The trouble arises here. Is it the same as *Oxytropis* and what is its type? If the genus had been based on a section of *Astragalus* in Linnaeus' *Species Plantarum*, one could apply the rule which states: "If a subgenus or section of a genus is raised to generic rank, the type of the subgenus or section remains the type of the new genus." The type could be selected from the two species best known to Linnaeus, either *Astragalus montanus* L., the first published, or *A. campestris* L., the only one endemic from Linnaeus' standpoint. In both cases it would be a typical species of *Oxytropis*, but this procedure cannot be applied, for *Aragallus* was not based on a section in the *Species Plantarum*, but in the 14th edition of the *Systema Vegetabilium*, where the Linnaean section had been modified, some species added and some excluded. It was no longer a section of Linnaeus but one of Murray, the author of that edition, and who can tell what species were best known to him or to Necker? *A. montanus* was no longer the first, but *A. verticillaris* which should have been in *Spiesia*, if Greene interpreted that genus correctly. Furthermore, the genus *Aragallus* is based wholly on a vegetative habit, the scapose subcaulescent habit of the plants, not on any structure of the flower or the fruit. *Oxytropis* DC., on the other hand, is well defined and, as far as the majority of the species are concerned, constitutes a very natural group of related species. The remaining few segregated off would constitute another natural group. *Aragallus*, besides, contained a mixture of types of which only 4 out of the 12, belong to the genus *Oxytropis*, the other 8 to *Astragalus* in a broad sense. It is hopeless to try to determine the type even if one knows what species were included in the genus *Aragallus*. The best one can do is to cite *Aragallus* as a synonym of *Oxytropis*, in part. When a stout defender of the American Code finds cases of this kind, he feels inclined to accept a list of *Genera Conservanda*.





RYDBERG: NORTH AMERICAN FABACEAE

DEVELOPMENT OF THE OVULE AND EMBRYO SAC OF ALFALFA

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The present investigation was carried on in connection with a study of microsporogenesis in alfalfa (*Medicago sativa* L.) already reported (19). The same kinds of material were used and the methods employed were similar to those given in the previous paper. Licent's fluid gave the best results with embryo sacs and was used more than any other fixing agent. The common and variegated alfalfas were found identical in this study also, and the data given in the present paper have been derived from the investigation of both types.

OBSERVATIONS

The Ovule

As is characteristic of the Leguminosae, the ovules arise in two rows, one on each side of the ventral suture. At first they are very crowded in the rows (Pl. XVIII, fig. 1) so that when cross-sections of the carpel are examined two ovules are apparently side by side (fig. 2). The carpel lengthens very rapidly, however, and the ovules soon become widely separated in the rows.

The direction of the curvature of the ovule may be determined mechanically by the growth of the carpel. The young ovule is orthotropous until it comes in contact with the dorsal wall of the carpel and then it begins to curve. This curvature is usually towards the base of the ovary (fig. 3), but occasionally one of the ovules at the stylar end curves towards the apex (figs. 1, 4). The carpel itself coils as a result of a curvature which is greater near the apical end. This coiling is caused by the more rapid elongation of the dorsal wall, and since the apex of the anomalous ovule just mentioned grows out against the wall, the curving of the carpel drags the tip of the ovule towards the stylar end and starts its growth in that direction. The direction having thus been determined, growth continues normally. The writer made no attempt to determine the cause of the normal downward curvature of the ovules, but whatever its nature, it is often overcome in one or more of the upper ovules by the mechanical influence exerted by the carpel wall.

There are two integuments in the young ovule. Each integument is composed of two layers of cells except near the micropyle, where the outer is considerably thicker (Pl. XIX, fig. 14). The inner integument appears before the outer, but the latter grows much more rapidly and soon covers

the former. The inner integument does not usually extend entirely over the nucellus. A considerable part of the nucellus around the micropyle is covered by the thickened border of the outer integument.

The funiculus becomes geniculate in shape and the ovule curved so that it becomes campylotropous, with the micropyle against the funiculus (fig. 20). The features of the ovule here described conform in a general way to the description given by Warming in his classic work (22) on the ovule of *Ribes*.

The Archesporium

The ovule first appears as a mound of tissue, and one or more archesporial cells beneath the epidermis can be distinguished by their large nuclei and dense cytoplasm (fig. 5). The number of these cells varies considerably. Occasionally there is only one, but more frequently two or more are found. Guignard (11) found only one archesporial cell in the megasporangium of *Medicago arborea*, but Martin (16) later reported a multiple archesporium in *M. sativa*. This has been reported in a number of genera of other families, being observed in *Rosa* by Strasburger (21), in *Salix* by Chamberlain (4), in *Quercus* by Conrad (6), in *Alchemilla* by Murbeck (18), and in *Callipeltis* by Lloyd (15). The archesporium in *Medicago sativa* is not sharply delimited from the other tissues, and it is therefore often difficult to determine with certainty whether a cell is archesporial or not. After the archesporial cells divide, however, it is easy to recognize several distinctly sporogenous cells (figs. 7, 9, 10).

The Sporogenous and Parietal Cells

The division of the archesporial cell gives rise to a primary parietal cell and a primary sporogenous cell (figs. 6, 7). The primary parietal cell is quite variable in its behavior. It may divide only by a transverse wall, as in figure 9; but longitudinal divisions may follow (fig. 8). Sometimes two transverse divisions occur and three parietal layers result, the cells of any of which may divide again. As a result of this procedure the sporogenous tissue is more or less deeply imbedded. Guignard (11) has shown that the sporogenous tissue of *Acacia*, *Cercis*, *Cytisus*, and *Lupinus* is deeply imbedded in the nucellus. Martin (16) has also shown considerable parietal tissue in *Vicia americana* and in three species of *Trifolium*.

Sporogenous cells have occasionally been found in a linear arrangement (fig. 9). Certain large cells of the nucellus are sometimes in alignment with the sporogenous cells, and it is then difficult to distinguish between the two types. Such a row of cells may easily be mistaken for megaspores, as Schaffner (20) has pointed out in his work on *Typha*. However, if the chromatin thread of the nucleus can be observed in synizesis (fig. 9), or some other stage peculiar to gonotokonts, the recognition of the megaspore mother cells as such is not difficult.

The primary sporogenous cell is rendered conspicuous by its greater

size and its enlarged nucleus. It is usually ovoid in shape, with the nucleus in the larger end (figs. 9, 10). The protoplasm of the sporogenous cell is not notably dense nor does it stain especially dark.

Formation and Development of the Female Gametophyte

The primary sporogenous cell develops directly into the megaspore mother cell without dividing again. This cell by two meiotic divisions gives rise to a tetrad of megaspores (fig. 11). As is usually the case in angiosperms, the three micropylar megaspores disintegrate and the chalazal one develops into the embryo sac (fig. 13).

This method of embryo-sac formation is common in the Leguminosae. Hérail (12), however, states that in *Medicago arborea* the axial subepidermal cell (our hypodermal cell) divides horizontally, the lower daughter cell becoming the subapical cell (our megaspore mother cell). He states that the subapical cell then grows rapidly and develops directly into the embryo sac. Young (24) reports another exception in *Melilotus alba*, claiming that the megaspore mother cell develops into the embryo sac without first undergoing tetrad division. On the other hand, Coe and Martin (5), in a later investigation of *M. alba*, showed that a first division of the megaspore mother cell does occur and that it results in the formation of two daughter cells. They state further that the development of the embryo sac proceeds in the ordinary way, *i.e.*, the inner megaspore persists.

As far as the writer has observed, the plane of the first division of the megaspore mother cell in *Medicago sativa* is at right angles to the long axis of the nucellus, but the plane of the second division is sometimes longitudinal (fig. 11), the latter condition being occasionally found in the terminal member of the diad. A tetrad of megaspores arranged in this way was observed by Mottier (17) in one of the Ranunculaceae, and more recently by Baranov (2) in *Drimopsis maculata*. Ducamp (9) reported various groupings of the megaspores in the tetrads of *Fatsia japonica*, but does not show the particular arrangement described here in *Medicago sativa*.

In the ovule of alfalfa, two or three tetrads are often found (Pl. XVIII, fig. 12), but only a single megaspore has ever been found to develop into a mature embryo sac. In extreme cases, the chalazal megaspores of as many as three tetrads can be observed to persist and undergo the first stages of development, but two of these megaspores disappear about the time of the two-nucleate stage. Martin (16) shows functional megaspores of two tetrads remaining after the other members have disintegrated, and one of the megaspores is in the two-nucleate condition. Hofmeister (13) found ovules of some species of *Rosa* with several embryo sacs, but states that egg-cells were found in only one embryo sac of each ovule. Andersen (1) has recently reported the development of two embryo sacs in *Poa pratensis* and *P. compressa*, and states that in these species more than one embryo sac with an embryo in each is of frequent occurrence. Jönsson

(14) states that in *Trifolium pratense* more than one embryo sac is formed, and that these develop to maturity and are often fertilized. Martin (16) later investigated *Trifolium pratense*, *T. hybridum*, and *T. repens* and found several sporogenous cells but did not find more than one tetrad in a nucellus.

In *Medicago sativa*, after the disintegration of the three micropylar megaspores the functional megaspore becomes round and enlarged. This usually occurs at about the time the outer integument grows beyond and encloses the inner. The functional megaspore is quite deeply imbedded in the nucellus (fig. 13), which is broken down and digested as the embryo sac develops. The nucleus of the functional megaspore divides and gives rise to the primary micropylar and the primary antipodal nuclei (fig. 14). These migrate respectively to the micropylar and antipodal ends of the embryo sac where they usually undergo two more divisions, and give rise first to four and then to eight nuclei (figs. 15, 17). While the embryo sac is in the two-nucleate stage, a large vacuole appears near the center. This soon becomes filled with starch and persists until all of the nuclear divisions in the embryo sac are complete. At this stage the embryo sac is slightly elongated and occupies all of the interior of the nucellus except the epidermis.

Occasionally only one nuclear division occurs in the chalazal end and six instead of eight nuclei are formed in the embryo sac. Figure 18 shows a six-nucleate embryo sac in which the micropylar nuclei have just completed their last division while the two chalazal nuclei show no sign of mitosis. Figure 16 shows the division of the four nuclei as it usually occurs. The embryo sacs shown in figures 17 and 18 are from ovules borne in the same ovary, and exhibit a surprising variation. Other embryo sacs have been found with seven nuclei, but their development was not followed.

In those embryo sacs containing eight nuclei, three nuclei in the chalazal end and three in the micropylar end become separated from the remainder of the embryo sac, and each nucleus lies in an individual mass of cytoplasm (fig. 19). This division of the cytoplasm occurs very rapidly after the process has been initiated, but the division was not followed in detail. The three cells at the chalazal end are the antipodal cells and the three at the micropylar end, after certain modifications, become the egg-apparatus. The two nuclei devoid of a limiting membrane become polar nuclei. One of these from the chalazal end moves to a position near its mate in the micropylar end, and the two come into contact near the egg-apparatus.

Chamberlain (4) has found the antipodal cells of *Salix*, also, to be of an exceedingly transitory character. Coulter and Chamberlain (7) state that antipodal cells of similar evanescent character are of common occurrence in various groups of angiosperms. Guignard (11) found that antipodal cells persist for a long time in the Mimosoideae and Caesalpinoideae, but that they disappear earlier in the Papilionoideae.

The Mature Female Gametophyte

The polar nuclei remain in contact for a considerable period of time in a region of dense cytoplasm near the egg-apparatus. Stages have been observed in which they had begun to fuse before fertilization of the egg-cell had taken place (fig. 21), but a complete fusion at this time has not been observed. It seems quite probable that the partial fusion of these nuclei is a result of delayed fertilization, because the embryo sac reaches complete maturity in all other respects considerably before fusion begins. At present, however, it is impossible to state whether this partial or delayed fusion is a normal occurrence, since fertilization in *Medicago* has not been studied.

The partial fusion of the two polar nuclei takes place while the chromatin is in the metabolic stage (fig. 21). At this time the cytoplasm of the embryo sac is very vacuolate except in the region about the egg-apparatus and the polar nuclei. Starch grains remain in these regions until the gametophyte is mature. The presence of starch in the embryo sac seems to be of very frequent occurrence in higher plants. Dahlgren (8) has summarized the literature on this subject and shows that the phenomenon occurs in a large number of species distributed among at least fifty-four families.

The cells of the egg-apparatus are pyriform and each is bounded by a *hautschicht*. The nuclei of the synergids are in the micropylar ends, and the nucleus of the egg-cell is in the distal end (fig. 20). All of these cells are vacuolated in the ends opposite the nuclei. The tissues of the nucellus at the micropylar end have now entirely disappeared and the embryo sac is in contact with the integuments. Since the inner integument does not extend entirely around the nucellus, the outer integument covers the embryo sac at the micropyle. The gametophyte is therefore in contact with the outer integument, the inner integument, and, at the chalazal end, with the outermost layer of the nucellus.

In *Medicago sativa* the nutritive material for the embryo sac is derived chiefly from the digestion and absorption of adjacent tissues, but certain partially specialized cells (fig. 20) also serve in the conduction of food to the embryo sac. These cells, which have been described by Ernst (10) in *Tulipa gesneriana* and by him termed "Leitzellen," are of rather striking appearance and extend into the chalazal end of the embryo sac. By the time the female gametophyte is mature, vessels also can be traced from the base of the funiculus to the Leitzellen.

The results of the present investigation of the embryo sac substantiate and supplement the work of Martin (16) who has figured and described certain stages in the development of the embryo sac of *Medicago sativa* in comparison with other species of the Leguminosae.

DISCUSSION

An unusual amount of variation has been found in the cytological features of the ovules of *Medicago sativa*. The variability of the Leguminosae as a group has already been shown by Guignard (11), who worked chiefly on embryological structures. The modifications described in the present paper, however, are more pronounced than those found by Guignard within any given species. There is extreme variation in the number of archesporial cells, in the number and arrangement of parietal and sporogenous cells, and in the form and number of tetrads.

Alfalfa is generally recognized as being a very variable plant; this variability is probably due to the fact that the plant has long been under cultivation. In addition, hybridization may have occurred. Westgate (23) and Brand (3) have suggested that Grimm alfalfa is a hybrid between *Medicago sativa* and the closely related species *M. falcata*. Their suggestions were based upon a study of the conditions under which the plant developed historically, together with studies of its flower color and of its gross morphological features. In the present investigation both Grimm and common alfalfa were studied and no consistent differences were found in the cytological characteristics of the two forms. Before conclusive statements can be made concerning the origin of Grimm alfalfa, however, a similar study of *Medicago falcata* should be made.

The earlier investigations of *Medicago* and closely related genera of Leguminosae indicated that megasporogenesis proceeds as in the typical case of *Lilium*; i.e., all four megaspores function. This method of megaspore formation was reported by Hérail (12) in *Medicago arborea*, by Guignard (11) in *Medicago arborea* and *Melilotus alba*, and by Young (24) in *Melilotus alba*. In more recent investigations, however, it appears that the process is the same as that usually found in the dicotyledons, in which one of the megaspores only is functional while the other three disintegrate. This has been shown by Martin (16) to be true for *Medicago sativa*, *Vicia americana*, *Trifolium pratense*, *T. hybridum*, and *T. repens*, and by Coe and Martin (5) for *Melilotus alba*. The present study furnishes additional data.

SUMMARY

1. As a rule, the curvature of the ovules in *Medicago sativa* is towards the base of the ovary, but occasionally, owing to the pressure exerted by the carpel wall, one of the upper ovules curves towards the stylar end.
2. The archesporium may consist of one or of several cells.
3. The amount of parietal tissue formed is variable, but it usually consists of a group of cells.
4. Several tetrads of megaspores may be formed, but only one embryo sac develops to maturity.
5. The chalazal megaspore develops into the embryo sac and the three micropylar megaspores disintegrate, as is usually the case in dicotyledons.

6. An eight-nucleate embryo sac is formed in the majority of the ovules, but sometimes only one nuclear division occurs in the chalazal end and a six-nucleate embryo sac results.

7. The antipodal cells disappear very early, long before the female gametophyte is mature.

8. Partial fusion of the polar nuclei was observed in embryo sacs in which fertilization of the egg-cell had not yet occurred; in no case was the fusion complete.

9. The embryo sac is long and somewhat curved at maturity.

10. The chief method of obtaining nutritive supplies for the embryo sac is by the digestion and absorption of adjacent tissues.

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DESCRIPTION OF PLATES

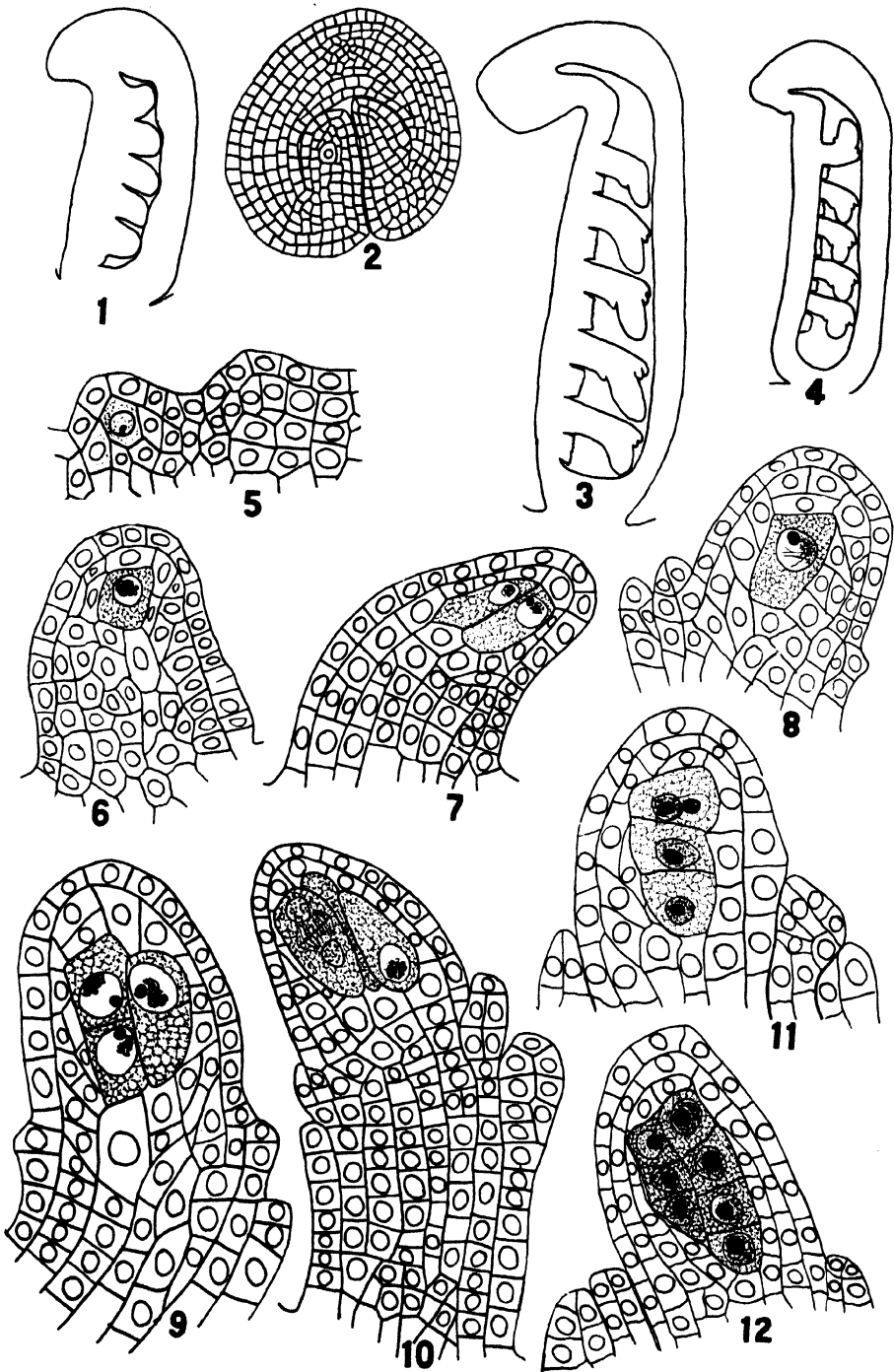
All of the figures were drawn with the aid of an Abbe camera lucida under Bausch and Lomb apochromatic objectives and compensating oculars. As several combinations of lenses were used, the approximate magnification of each figure is given.

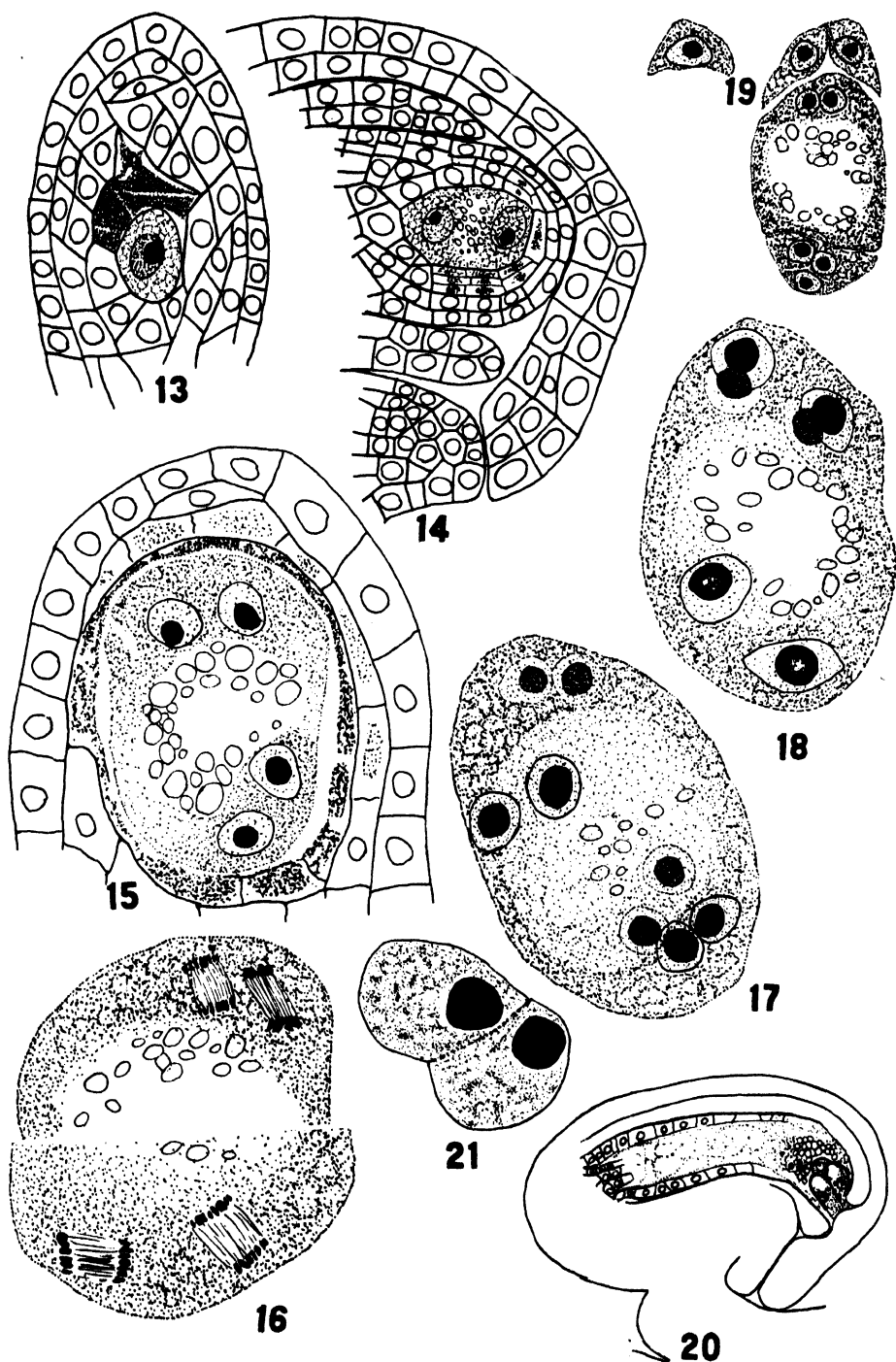
PLATE XVIII

- FIG. 1. Young carpel. Longitudinal section. Tips of ovules pressed against carpel wall. $\times 66$.
- FIG. 2. Very young carpel. Cross section. Carpels in two rows. $\times 66$.
- FIG. 3. Later stage. Ovules curving towards base of carpel. $\times 50$.
- FIG. 4. Upper ovule curving towards apex of carpel. $\times 50$.
- FIG. 5. Mounds of tissue on border of carpellary leaf which develop into ovules. Archesprial cells beginning to differentiate. $\times 280$.
- FIG. 6. Later stage. Archesprial cell has divided giving rise to primary parietal cell and primary sporogenous cell. $\times 280$.
- FIG. 7. Two sporogenous cells in nucellus. $\times 280$.
- FIG. 8. One sporogenous cell; three parietal cells. $\times 280$.
- FIG. 9. Three sporogenous cells; two in linear arrangement. $\times 420$.
- FIG. 10. Three sporogenous cells in lateral arrangement. $\times 420$.
- FIG. 11. Tetrad completely formed. Lateral arrangement of the two apical megaspores. $\times 420$.
- FIG. 12. Two tetrads of megaspores in a nucellus. Only three megaspores of one tetrad showing; fourth not in focus. $\times 420$.

PLATE XIX

- FIG. 13. Three of the megaspores disintegrating. $\times 420$.
- FIG. 14. Two-nucleate stage of embryo sac. Inner integument does not close at micropyle. Outer integument covers nucellus. $\times 280$.
- FIG. 15. Four-nucleate embryo sac. Starch grains present. $\times 700$.
- FIG. 16. Nuclear division following the four-nucleate stage. The two ends of the embryo sac were found on adjoining sections. $\times 1000$.
- FIG. 17. Eight-nucleate embryo sac. $\times 1000$.
- FIG. 18. Six-nucleate embryo sac. Only one division occurred in the chalazal end. $\times 1000$.
- FIG. 19. Three nuclei of the micropylar end and three of the chalazal end separated by a division of the cytoplasm. The two polars have come in contact. $\times 660$.
- FIG. 20. Embryo sac ready for fertilization. $\times 100$.
- FIG. 21. Polar nuclei fusing before entrance of male nuclei. $\times 1600$.





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CHROMOSOME STABILITY IN THE GENUS *RHODODENDRON*

KARL SAX

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Rhododendron is a very polymorphic genus consisting of four subgenera and eight sections, and includes species formerly classed in different genera. According to Millais (1) there are about 460 species distributed as follows: 280 in China and Japan, 46 in British India, 62 in Malaya, 46 in the Orient, 1 in Australia, 4 in Europe, 3 in Siberia, 2 in the Arctic, and 17 in North America. Only a few species of the true *Rhododendron* are found in America but the *Azalea* group is fairly well represented.

Beginning early in the last century numerous hybrids have been made until now most of the many garden varieties are of mixed and doubtful parentage. The varieties of the true *Rhododendrons* (*Eurhododendron*) of most horticultural value have been derived from crosses involving the American species *R. catawbiense* and *maximum* and the Old World species *caucasicum*, *arboreum*, and *ponticum*, and are generally known as Catawbiense hybrids. The Chinese species *R. discolor* and *Fortunei* have also been used as parents of valuable garden varieties.

In the *Azalea* group hybridization has been confined mostly to the section *Pentanthera*. The species most commonly used as parents include the American *Azaleas* *R. calendulaceum*, *nudiflorum*, *viscosum*, and *occidentale*, the Oriental species *molle* and *japonicum*, and the Eurasian species *luteum*.

Crosses between different sections of subgenera have seldom been made, although as early as 1838 a cross between *R. luteum* and *R. canadense* was described. Recently a cross between the same sections (*Rhodora* and *Pentanthera*) has been made by Fraser who crossed *R. japonicum* with *R. canadense* (4).

The first recorded hybrids in the genus were between different subgenera. According to Wilson and Rehder (4) the first *Rhododendron* hybrid known was between *R. ponticum* and an *Azalea* species. It originated at Thompson's Nursery near London before 1800. Since then a number of hybrids have been made between *Eurhododendron* and *Anthodendron* but they have not been of much horticultural value and none is represented in the Arboretum.

[The Journal for March (17: 187-246) was issued March 20, 1930.]

The great majority of *Rhododendron* hybrids have been made between species in the same section. Apparently crosses between different subgenera and sections are usually difficult or impossible to make.

Representations of the more important subgenera and sections of the genus are grown in the Arnold Arboretum. Cytological studies have been made to determine the relation between chromosome number and taxonomic classification and to observe chromosome behavior in the known hybrids.

All chromosome counts are from permanent smears of pollen mother cells which were fixed in Nawaschin's solution and stained with crystal violet iodine. The reduction divisions of *Rhododendrons* begin late in August and may be found in some species until the middle of October.

Following are the species investigated, with references to the figure numbers in Plate XX, the chromosome number, and the native habitat.

Subgenus 1. *Eurhododendron*

| | | |
|---------------------------------|----|---------------|
| Sec. 1. <i>Leiorhodion</i> | | |
| <i>R. catawbiense</i> (1)..... | 13 | North America |
| <i>R. maximum</i> (2)..... | 13 | North America |
| Sec. 2. <i>Lepipherum</i> | | |
| <i>R. carolinianum</i> (3)..... | 13 | North America |
| Sec. 4. <i>Rhodorastrum</i> | | |
| <i>R. dauricum</i> | 13 | Korea, Japan |

Subgenus 3. *Anthodendron*

| | | |
|---|----|---------------|
| Sec. 1. <i>Tsutsutsi</i> | | |
| <i>R. obtusum japonicum</i> (4)..... | 13 | Japan |
| <i>R. obtusum Kaempferi</i> | 13 | Japan |
| <i>R. yedoense poukhanense</i> (5)..... | 13 | Korea |
| Sec. 2. <i>Sciadiorhodion</i> | | |
| <i>R. reticulatum</i> | 13 | Japan |
| <i>R. Schlippenbachii</i> (6)..... | 13 | Korea, Japan |
| Sec. 3. <i>Rhodora</i> | | |
| <i>R. Vaseyi</i> (7)..... | 13 | North America |
| <i>R. canadense</i> (8)..... | 26 | North America |
| Sec. 4. <i>Pentlanthera</i> | | |
| <i>R. japonicum</i> (9)..... | 13 | Japan |
| <i>R. roseum</i> (10)..... | 13 | North America |
| <i>R. viscosum</i> (11)..... | 13 | North America |
| <i>R. calendulaceum</i> (12)..... | 26 | North America |
| <i>R. arborescens</i> | 13 | North America |

The subgenera *Azaleastrum* and *Therorhodion* are represented by only one species each according to Rehder's Manual (2) and neither of these is grown in the Arnold Arboretum.

Considering the great variation and geographic range of the *Rhododendron* species it is surprising to find the chromosome number so constant.

SPECIES HYBRIDS

A considerable number of *Rhododendron* hybrids is grown in the Arboretum but in most cases the exact parentage is not known. In certain cases the parents are known but the hybrid may be a second generation segregate. This is especially true of the garden varieties of known hybrids. In a few cases, however, the entire history of the hybrid is available. If species hybrids produce fertile seeds in F_1 it is probable that there is complete or almost complete pairing of chromosomes so that in any case the cytological examination is of some value in determining the parental chromosome numbers.

A plant labeled as a *R. Smirnovii* hybrid is probably a cross of *R. Smirnovii* with a Catawbiense hybrid. The hybrid is intermediate in morphological characters. At the first reduction division there are 12 bivalents and 2 univalents. The chromosomes at the first telophase are shown in figures 13A and 13B where 12 chromosomes are at each pole and the two univalents between.

R. praecox is a cross between *R. dauricum* and *R. ciliatum*. The variety "Early Gem" is probably a segregate from this cross. It has 13 pairs of chromosomes at the first reduction division (Fig. 14).

R. laetevirens is supposed to be a hybrid between the American species *carolinianum* and the European species *ferrugineum*. In the hybrid 12 bivalents and 2 univalents were found at diakinesis (Fig. 15).

R. purpureum is one of the so-called Catawbiense hybrids of unknown origin, but its parents include *R. catawbiense* and *maximum* or *ponticum*. In some pollen mother cells there are 13 pairs of chromosomes but frequently there are 12 pairs and 2 univalents at the first metaphase of the reduction divisions (Fig. 16). A similar hybrid known as *R. perspicum* has 13 gametic chromosomes (Fig. 17).

In the *Azalea* group numerous hybrids have been made, especially in the section Pentanthera. In 1915 Mr. W. H. Judd crossed *R. occidentale*, which is found in Oregon and California, with *R. calendulaceum*, a native of the eastern United States. This is a cross of a diploid and a tetraploid form. In the F_1 hybrid there are about 26 chromosomes at the telophase of the first reduction division of which about half are apparently univalents (Fig. 18).

Mr. Judd also crossed *R. occidentale* with *R. japonicum*, a native of Japan. The parental chromosomes are completely compatible in F_1 reduction divisions. The 13 paired chromosomes at late diakinesis are shown in figures 19 and 21.

According to Rehder (2) *R. viscosum* is a cross between *R. molle* from China and *R. viscosum* from America. The cross was made in England before 1842. Rehder's determination of the parents of this hybrid is based on taxonomic comparisons. Evidently both parents had 13 pairs of

chromosomes and in F_1 there are about 12 paired and 2 single chromosomes at early metaphase (Fig. 20).

The *gandavense* hybrids are a collective group which includes all hybrids between American Azaleas and the Eurasian *R. luteum* and which are of mixed and uncertain parentage. One of these hybrids in the Arboretum seems to be completely fertile and the chromosome behavior is entirely normal. The 13 pairs of chromosomes at the metaphase of the first division are shown in figure 22.

One of Waterer's crosses between *R. molle* from China and *R. occidentale* from California is known as *R. albicans*. This hybrid shows complete compatibility of the parental chromosomes, as shown in figure 23.

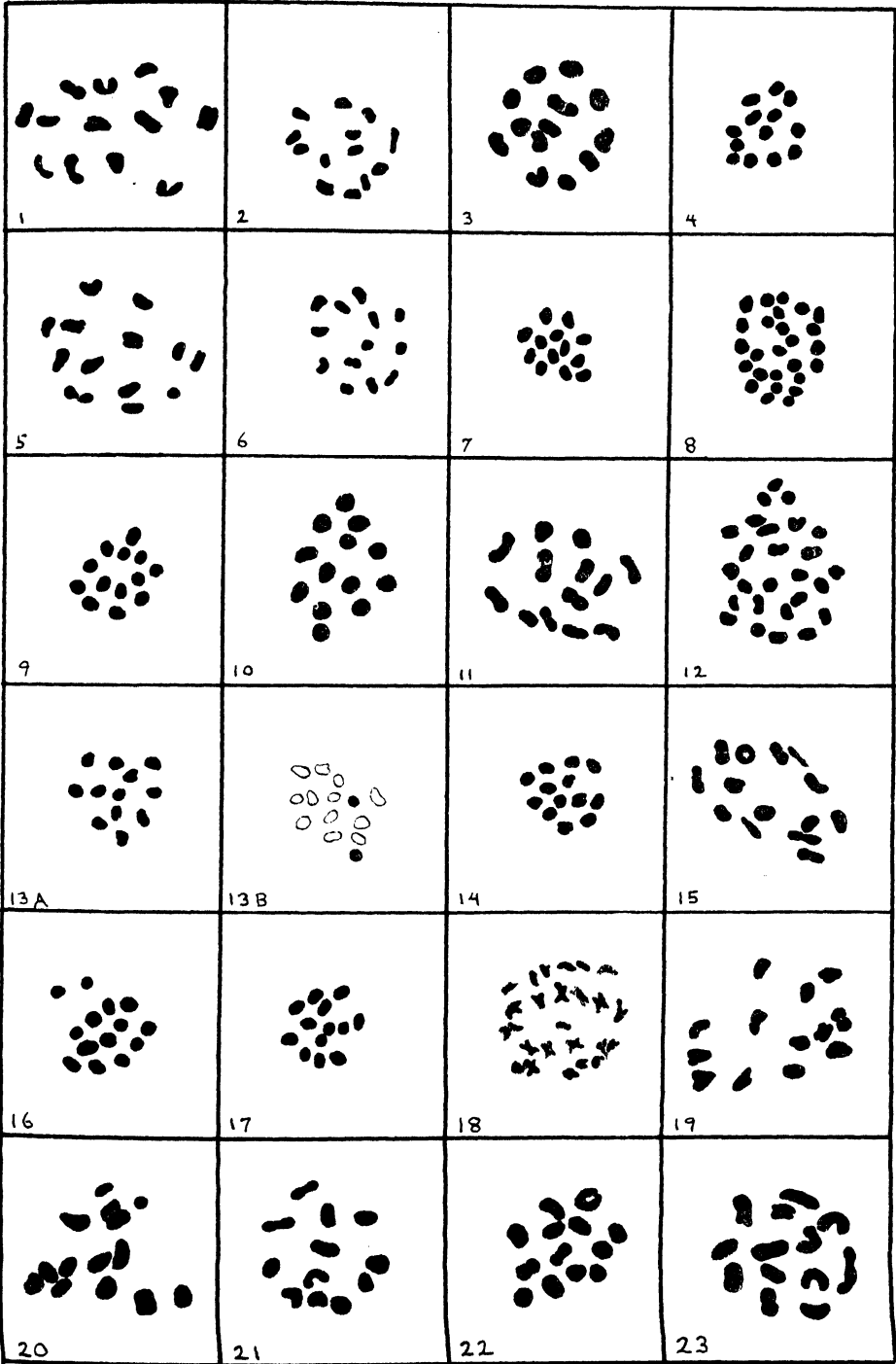
The closely related species of *Rhododendron* when crossed produce hybrids which are usually fertile, judging from the large number of hybrid varieties. Some of the hybrids studied cytologically may be F_2 segregates, but the fact that such segregates can frequently be obtained indicates that chromosome compatibility must exist in F_1 hybrids. In several cases the exact history of the hybrids is known and in such hybrids there is complete or almost complete compatibility of the parental chromosomes in F_1 . The hybrids between Oriental and American species are of most interest as they show how stable the chromosomes are in their genetic constitution.

CHROMOSOME STABILITY

The *Rhododendrons* undoubtedly originated in eastern Asia where most of the species are now found. The American species must have migrated from Asia by way of the land bridge formerly connecting Asia and America. According to Scharff (3) there was direct land migration between Asia and North America in early and late Tertiary or at any rate in Pre-Pliocene times. The disappearance of the land connection and the beginning of the Ice Age in North America effectively blocked further plant migration. In view of the slow rate of plant migration and the great length of time since such migration was possible it is probable that Oriental and American species of *Rhododendrons* have been separated for millions of years. Over this long period of time most of these species have not undergone any change in chromosome number and, still more remarkable, the chromosomes of certain species have not changed fundamentally in their genetic constitution. The fact that there is complete or almost complete pairing of chromosomes in hybrids between Oriental and American species indicates that in *Rhododendrons* at least the chromosomes are very stable in their genetic constitution over very long periods of time.

SUMMARY

Chromosome counts of representative species of *Rhododendron* show that 13 is the fundamental chromosome number in the genus and this number is found in both the true *Rhododendrons* and in the Azaleas. Two tetraploid



species were found but tetraploidy is not characteristic of any one subgenus or section. Although the genus is very polymorphic the chromosome numbers of representative species are very uniform.

Cytological studies of certain hybrids between Oriental and American species show that there is complete or almost complete compatibility of the parental chromosomes in the hybrids, although the parents have probably been separated for millions of years. These results indicate that the chromosomes of *Rhododendron* are very stable in their genetic constitution.

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DESCRIPTION OF PLATE XX

All drawings were made from permanent smears of pollen mother cells. Magnification about 2500 X.

- FIG. 1. *Rhododendron catawbiense*. Diakinesis.
- FIG. 2. *Rhododendron maximum*. Telophase, 1st division.
- FIG. 3. *Rhododendron carolinianum*. 1st metaphase.
- FIG. 4. *Rhododendron obtusum japonicum*. 1st metaphase.
- FIG. 5. *Rhododendron yedoense poukhanense*. Diakinesis.
- FIG. 6. *Rhododendron Schlippenbachii*. Telophase, 1st division.
- FIG. 7. *Rhododendron Vaseyi*. Telophase, 1st division.
- FIG. 8. *Rhododendron canadense*. 1st metaphase.
- FIG. 9. *Rhododendron japonicum*. 1st metaphase.
- FIG. 10. *Rhododendron roseum*. 1st metaphase.
- FIG. 11. *Rhododendron viscosum*. Diakinesis.
- FIG. 12. *Rhododendron calendulaceum*. Metaphase. 2d division.
- FIG. 13, A, B. *Rhododendron Smirnovii* hybrid. 1st telophase. 12 chromosomes at each pole with two lagging univalents.
- FIG. 14. *Rhododendron praecox* "Early Gem" (*R. dauricum* × *R. ciliatum*). 1st telophase.
- FIG. 15. *Rhododendron laevis* (*R. carolinianum* × *R. ferrugineum*). Diakinesis.
- FIG. 16. *Rhododendron purpureum* (maximum hybrid). 1st metaphase. 12 bivalents and 2 univalents.
- FIG. 17. *Rhododendron perspicuum* (Catawbiense hybrid). 1st telophase.
- FIG. 18. *Rhododendron occidentale* × *calendulaceum* F₁. 1st telophase.
- FIG. 19. *Rhododendron occidentale* × *japonicum* F₁. Late Diakinesis.
- FIG. 20. *Rhododendron viscosesepalum* (*R. viscosum* × *R. molle*). 1st metaphase.
- FIG. 21. *Rhododendron occidentale* × *japonicum* F₁. Late Diakinesis. 12 bivalents and 2 univalents.
- FIG. 22. *Rhododendron gandavense* (*R. luteum* × *R. Mortieri*). 1st metaphase.
- FIG. 23. *Rhododendron albicans graciösa* (*R. molle* × *occidentale*). Late Diakinesis.

ON THE DEVELOPMENT AND MORPHOLOGY OF THE LEAF OF THE BANANA (*MUSA SAPIENTUM* L.)¹

ALEXANDER F. SKUTCH

(Received for publication November 15, 1929)

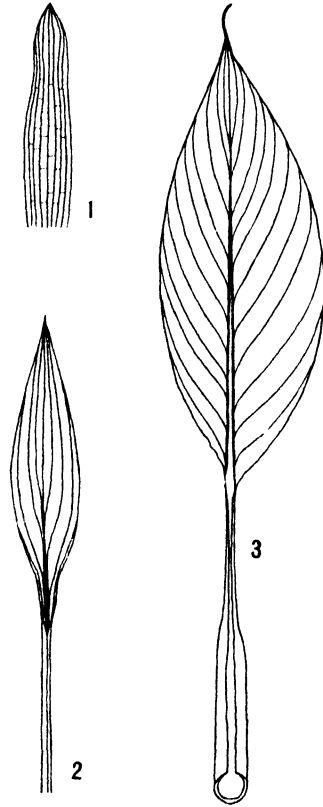
In a previous publication (18), I described the anatomy of the mature leaf of the banana. The process of development was considered only in reference to a few isolated points, such as the disruption and occlusion of the protoxylem elements, and a discussion of the morphology of the leaf was avoided. Many details, however, connected with the process of unfurling and the acclimatization, so to speak, of the young leaf to its environment, are meaningless unless we understand the past history of the leaf and its development while it was still hidden away inside the massive false-stem. During a recent sojourn in Panama, on a plantation where an abundance of material of many species and varieties of bananas was available, I was able to follow the early stages in the formation of the leaf. As a result, certain points which had previously been obscure, such as the presence of the fugacious hyaline wing which borders the newly unfurled leaf, the tearing off of the apex of the right half of the lamina in unfurling, and the basal inequality of the lamina, are now full of significance. These points can be best interpreted in the light of the phyllode theory, propounded by Mrs. Arber (4), and the banana, in turn, deserves to stand as a strong support of that theory, and to offer further confirmation from developmental history of a view which at present rests largely upon anatomical evidence. I trust also that it may prove a stepping stone to a better understanding of the development of the leaves of palms, those most interesting and complex of all monocotyledonous leaves.

SEEDLING LEAVES

The seed and seedling of the banana have been described by Greve (11) and others. It is not my purpose to go into that history here, but merely to figure and describe the sequence of leaves produced by the young plant. Text figure 1 shows the first plumular leaf of a hybrid seedling of *Musa sapientum*. It will be noticed that the leaf is represented by sheath alone, that the veins are all parallel and run without converging almost or quite to the tip of the leaf and that horizontal anastomoses occur between them. Succeeding leaves show a differentiation into lamina and sheath, and at length a petiolar portion is intercalated between these parts. The lamina is at first devoid of a distinct midrib; later one appears at the base, and in succeeding leaves approaches nearer and nearer to the apex. The leaf shown

¹ Botanical Contribution from the Johns Hopkins University no. 108.

in text figure 2 has a midrib only at the base, and in its venation resembles the leaves of many of the Liliaceae—*Convallaria*, for example. Text figure 3 represents a later leaf which, in the termination of the midrib in the apical third, and the direction of the lateral veins, might pass for the lamina of *Strelitzia reginae* among the Musaceae. A small precursory appendage is already present. As in successively older leaves the midrib approaches

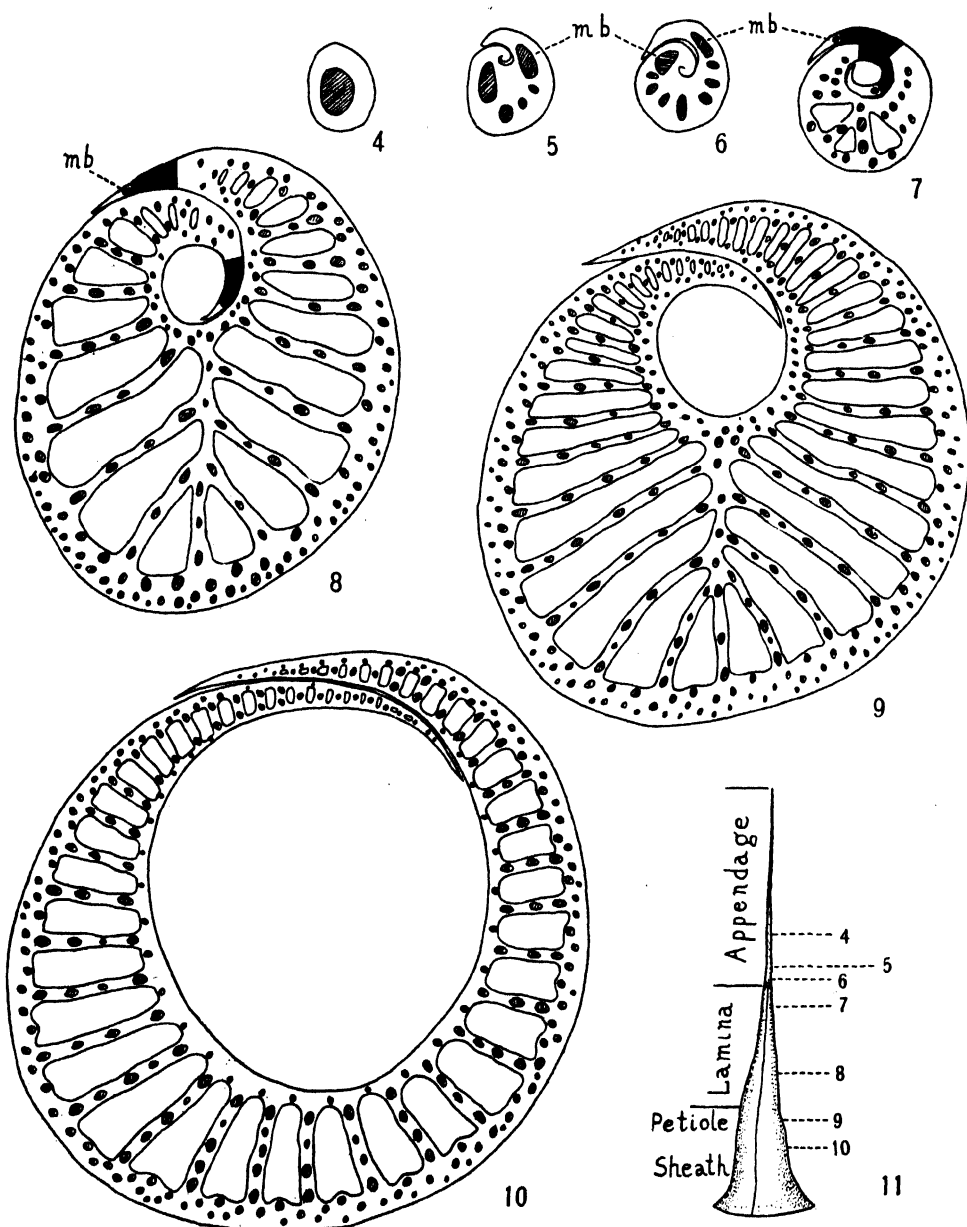


TEXT FIGS. 1-3. Leaves of seedling bananas (*M. sapientum* hybrid). 1. First plumular leaf, $\times 1\frac{1}{3}$. 2. A later leaf, $\times 1$. 3. Still later leaf from older seedling, $\times 1/3$.

nearer and nearer the apex, the veins diverge from it at greater and greater angles, until finally those in the central portion of the mature leaf stand at right angles to it, while at the base they are actually inclined backward (text fig. 17). This very gradual production of leaves of the mature type seems to indicate a long phylogenetic history, and its significance will be discussed in a later portion of this paper.

DEVELOPMENT OF THE MATURE LEAF

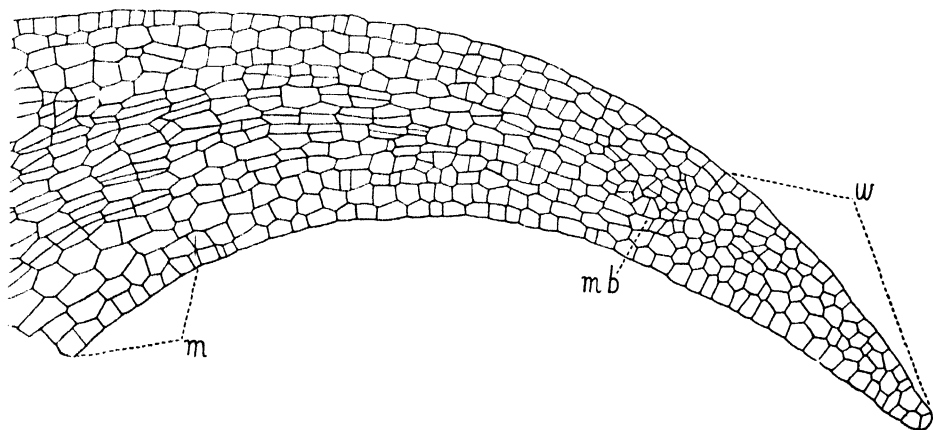
In the mature leaf four portions may be distinguished (18); these are: (1) The sheath, crescentic in cross-section, which, closely embracing and in turn embraced by its neighbors, helps form the peculiar false-stem of the



TEXT FIGS. 4-11. Figures 4-10 are diagrammatic cross-sections based on camera lucida drawings through the 52-mm.-long leaf rudiment shown in figure 11, at the levels indicated there. They are intended to show the degree of differentiation of tissues which is evident at this stage. The young lamina-halves (FIGS. 7 and 8) are indicated in solid black, the incipient vascular bundles by shading; the large unshaded spaces in the figures are the regions of the developing lacunae. *mb*, marginal vascular bundle. FIGS. 4-8 $\times 18$; FIGS. 9-10 $\times 12$; FIG. 11 $\times 1$. In these sections, which are represented with the acroscopic surface upward, the right lamina-half appears to cover the left. The leaf is, however, rolled in the normal direction, with the left half outermost.

banana plant; (2) the massive petiole; (3) the lamina; and (4) the precursory appendage or forerunner tip, a temporary organ which dies and usually breaks off shortly after the unfurling of the lamina (see text figs. 14-17). As I have already pointed out, these divisions are purely topographical, are useful merely for convenience in reference, and are valueless from the point of view of comparative morphology.

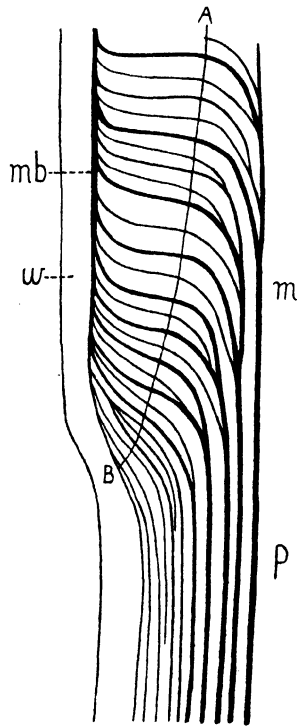
The leaves arise from the depressed growing point at the apex of the massive, bulbous rhizome. In order to reach the young rudiments, it is necessary first to strip away all of the sheaths of the mature leaves, which collectively form the false-stem, and then carefully cut away each of the immature leaves in order to expose the next younger, which is completely enclosed by it. As smaller and smaller leaf rudiments are uncovered, it is found that the precursory appendage occupies a larger and larger proportion of its length, until in the very young rudiments surrounding the growing point it accounts for about half the length of the leaf (table 1). Text figures 4-10 represent a series of cross-sections through a leaf rudiment 5.2 cm. long, taken from a large sucker which had not yet begun to "shoot," or produce flower-buds. The rudiment tapers so rapidly from base to apex that it was found necessary to reproduce the sections through the base on a smaller scale than that employed for the sections in the apical portion. The levels at which the hand-sections were cut are indicated in the drawing of the whole rudiment (text fig. 11). In all of what we may call the axial portion of this rudiment—sheath, petiole, midrib, and appendage—the entire ground plan of the mature organ is already laid down. Thus, in text figures 4-10, one may see the incipient vascular bundles, and the regions where the lacunae are beginning to form are already clearly differentiated from the surrounding walls and septa. In text figures 7 and 8, through the



TEXT FIG. 12. Cross-section through the left lamina-half of a leaf-rudiment 37 mm. in total length. *M*, midrib; *mb*, marginal vascular bundle; *w*, hyaline wing. The portion between the midrib and the marginal bundle is the young lamina-half. Camera lucida sketch $\times 220$.

region of the future lamina, however, it will be observed that the lamina-halves, which are indicated here in solid black, occupy an altogether insignificant portion of the whole cross-section, while in leaves which are just appearing from the top of the false-stem, and are already full grown, the left or outer half makes 4-5 turns about the midrib, and the right half 12 or more turns coiled in the concavity of its upper surface (text fig. 18).

Examined under a higher power of the microscope, the cells in the regions indicated by solid black in text figures 7 and 8 are found to be smaller and more deeply staining, in other words more highly meristematic, than those in the remaining portions of the cross-section. This region of young and very actively dividing cells does not extend quite to the margin of the



TEXT FIG. 13. Basal end of the left lamina-half of a rudiment 37 cm. long, cleared in xylol. A-B, boundary between midrib (M) and lamina-half; Mb, marginal vascular bundle; p, petiole; w, hyaline wing. Camera lucida sketch $\times 11$.

organ, but is bordered on the exterior by a region of cells which contain a less dense protoplasm and give evidence of greater maturity. This is the future hyaline wing of the lamina. Separating the wing from the lamina-half is the rudiment of the future marginal bundle of the lamina (mb, text figs. 7, 8, 12, and 13). In studying sections such as these, one is drawn to the conclusion that the lamina-halves are younger than the remaining portions of the leaf, and are intercalated between the midrib on the one hand, and the marginal bundle and wing on the other. In rudiments somewhat younger

than that chosen for illustration the lamina-halves are not evident, and the midrib tapers gradually to a thin margin which has already begun to become membranaceous. Here it may be seen that the marginal bundle of the lamina is merely the outermost bundle of the midrib, a continuation of the outermost bundle of the sheath, and completely homologous with the other bundles of midrib and sheath. The actual size of the rudiment when the lamina-halves first become evident varies somewhat with the size of the plant from which it is taken. Thus, the rudiment from which text figure 12 was drawn was only 37 mm. in total length, and already shows the origin of the lamina, while in a 44-mm. rudiment from a larger plant with a false-stem 3.1 m. high (table 1) the lamina could not be distinguished.

TABLE 1. *Measurements of the Immature Leaves of a Plant of Musa sapientum Subspecies seminifera with a False-stem 310 cm. High, with Four Mature Leaves for Comparison*

| Leaf No. | Condition | Total Length cm. | L. of Appendage cm. | L. of Lamina cm. | L. of Petiole cm. | L. of Sheath cm. | Breadth of Left Lamina-Half † cm. | Ratio, L. of Lamina: B. of Left Half |
|----------|---------------|------------------|---------------------|------------------|-------------------|------------------|-----------------------------------|--------------------------------------|
| 1 | Mature | 503*+ | Withered | 165 | 58 | 280 | 32.0 | 5.2 |
| 2 | " | 542+ | " | 177 | 68 | 297 | 31.4 | 5.6 |
| 3 | " | 544+ | " | 177 | 57 | 310 | 33.2 | 5.3 |
| 4 | " | 561+ | " | 192 | 62 | 307 | 32.0 | 6.0 |
| 5 | Just emerging | 383+ | Curled | | | | | |
| 6 | Immature | | | | | | | |
| 7 | " | 177 | 13+ | 197 | 173 | | 33.2 | 5.9 |
| 8 | " | 85 | 19 | 95 | 63 | | 15.9 | 6.0 |
| 9 | " | 38.8 | 18 | 42.5 | 24.5 | | 2.9 | 14.6 |
| 10 | " | 19.2 | 10.8 | 18.5 | 9.5 | | 0.4 | 46.3 |
| 11 | " | 9.2 | 9.1 | 5.9 | 4.2 | | 0.09 | 65.6 |
| 12 | " | 4.4 | 4.9 | 2.1 | 2.2 | | 0.05 | 42.0 |
| | " | 2.4 | 2.2 | 1.1 | 1.1 | | 0.00 | |
| | " | | 1.1 | | | | 0.00 | |
| | | | | | 1.3 | | | |

* Does not allow for the withered appendage.

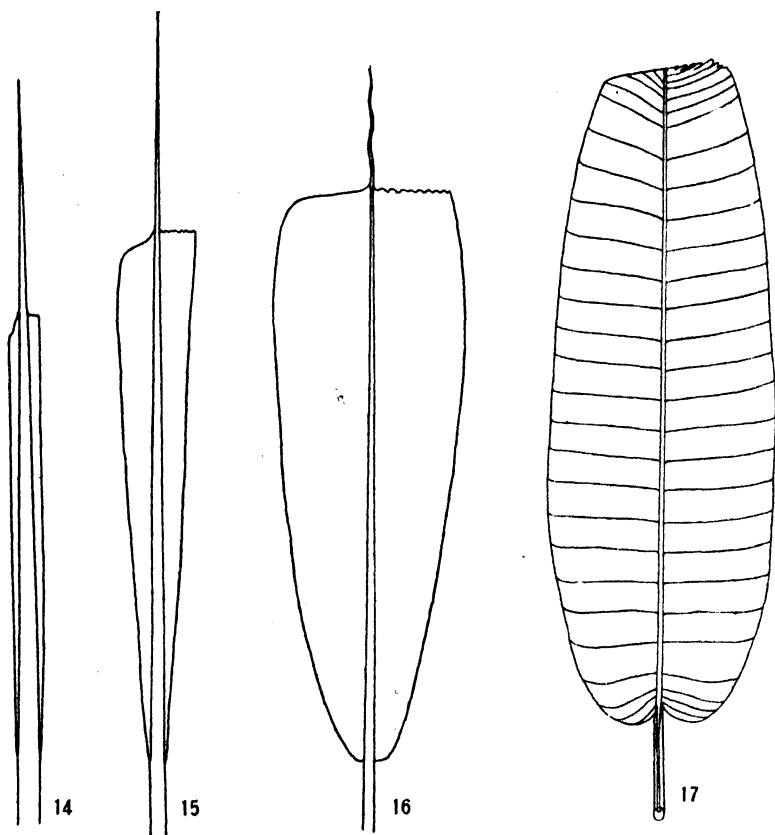
† The two sides of the lamina are very nearly equal.

The condition of tissues at the base of the lamina confirms the view derived from the study of cross-sections. Text figure 13 gives the appearance of the region at the base of the left ² or outer lamina-half of a rudiment 37 cm. long, which has been cleared in xylol and is viewed as a transparent object. The broad, membranaceous wing *W* is continuous from the petiole to the lamina, but has been pushed out above the point marked *B* by the intercalation of the lamina-half between it and the midrib to the right of the line *A-B*. As the leaf grows in breadth and the basal lobes begin to push downward (cf. text fig. 17), transverse wrinkles will appear at *B*, indicating that the development of this wing is not harmonious with that of the lamina-half. The marginal bundle is also continuous from the petiole to the lamina, but becomes stronger as it advances toward the apex, and is

² As reckoned by an observer standing at the petiole and looking toward the apex of the leaf in its normal orientation.

augmented by the distal ends of the veins of the lamina. The course of these is sufficiently evident from the figure, where it will be seen that the more distal portions of the lamina are supplied by the more central, and older, bundles of the midrib.

As the lamina-halves grow in breadth, they begin to assume the well-known convolute form of vernation. The left or outer half coils around the midrib and the right half, making finally 4-5 turns about them. How the thin, soft lamina-half manages to push between the midrib and the inner surface of the next older leaf, which fits tightly against it, without becoming



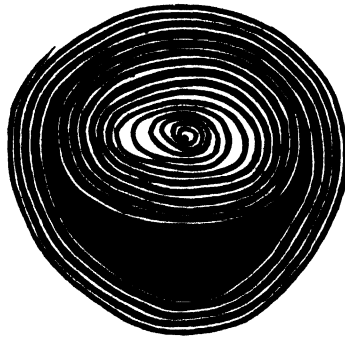
TEXT FIGS. 14-17. FIG. 14, lamina and appendage of Leaf 8 (see table 1) drawn to scale $\times 4/15$. FIG. 15, Leaf 7 $\times 2/15$. FIG. 16, Leaf 6 $\times 1/15$. FIG. 17, a mature leaf $\times 1/30$.

crumpled, without even the extremely delicate wing becoming folded back upon itself, remains a mystery to me. The outer surfaces of all the organs are covered by a smooth cuticula at a very early age, which eases, but does not remove, the difficulty of picturing the mechanics of the process. The inner half becomes coiled into the concavity of the upper surface of the midrib, making as many as 18 turns in the broadest portion of very large

leaves. Here it grows into a free space created by the enlargement of the midrib.

The intercalation of the lamina between the midrib and the wing begins at the apex and proceeds basally. As a result the laminae of young leaves are broader at the apex than at the base. Text figures 14-17, drawn to different scales so as to make all of approximately the same size, show this progressive change of shape. The earlier figures (14-16) must be considered as merely diagrammatic, since the shape of the midrib is such that it is impossible to spread the young lamina out in a plane. The lamina-halves at this stage are so thin and brittle that it is impossible to unroll them individually without first killing the cells and destroying their turgor by immersion in boiling water. As the leaf increases in breadth, the outer portions of each lamina-half grow more in length (that is, in the direction parallel to the margin), than the inner portion, the longitudinal growth of which is controlled by the midrib to which it is attached. This results in the pushing backward of the basal veins and in giving the lamina a more or less cordate base.

Not only is the apex of the lamina older than the base, but the differentiation of the marginal portions precedes that of the regions nearer the midrib. The order of the differentiation of the vascular bundles in the



TEXT FIG. 18. Diagrammatic cross-section of the lamina of a large leaf just before unrolling, to show the form of vernation.

various regions of the lamina was determined by injecting the leaf with eosin, since only those veins capable of conduction would show the stain. A whole plant was dug up in the early morning, while the dew was still heavy on it, all but the upper portion of the rhizome to which the leaves were attached was pared away under water, and this submerged in a vessel of 1-percent aqueous eosin solution. The solution rose into the completely enclosed leaves in response to the growth-requirements of their tissues. The plant was left in the solution for 6 to 22 hours, the object of the long periods being to allow the injection of all the vessels capable of conduction. In very young rudiments, only 6 cm. in length, in which the lamina was in the earliest stages of development, the tracheids of the precursory appendage

were stained densely to the apex, thus confirming the conclusions of Genter (9) and Raciborski (16) on the early maturity of this portion of the leaf. Only the most apical veins of the youngest leaves in which the lamina-halves were discernible indicated by taking the stain that they were functional in conduction. The marginal bundles became stained from the apex downward, and generally showed staining for a short distance below the most basal of the stained veins, indicating the basal flow of the solution in them. Many of the veins, especially those of the subordinate type, became stained for a greater or less distance inward from the marginal bundle, but not as far as the midrib. At the time the full-grown lamina begins to appear at the apex of the false stem, many of the subordinate veins still show the same inward course of water in their vessels.

Thus the marginal bundles are of importance in supplying water to the tissues of the growing lamina-halves, although they are apparently of little or no value to the leaf after it unfolds and transpiration begins. In the very young rudiment, the water enters these bundles through the precocious precursory appendage. As I have shown in a previous paper (18) and as is evident from text figures 4-8, the marginal bundles become the principal vascular supply of this appendage, while in its distal portion all of its bundles fuse together, so that here the marginal bundles are united with the central bundles which lead the water up from the rhizome through the sheath and midrib. Flowing backward from the apex in the marginal bundles, the water enters the more mature marginal extremities of the transverse veins in the lamina-halves, and proceeds inward along these. The tissues supplied by these veins accordingly receive their moisture in a very indirect manner. As soon as one of the veins has become capable of conduction throughout its length, it forms a short cut by which water can enter the marginal bundles by a more direct path than by way of the appendage, and this happens at a very early age, when the entire leaf-rudiment is only about 13 cm. long, and the lamina-halves still only a few millimeters broad.

In its basipetal direction of differentiation, the leaf of the banana agrees with those of most palms (7, 19), with the exception of *Chamaedorea*. In these the folding or invagination of the lamina to form the future pinnae usually proceeds from the apex downward.

At the time of its first appearance at the apex of the false-stem the lamina is full grown. It is still tightly rolled, and is quickly pushed upward and outward by the elongation of the sheath at its base, which up to this time has lagged behind it in growth, but is destined to exceed it in final length (see table 1). When it has emerged to practically its full height, the lamina begins to unroll. I shall discuss in two subsequent papers the emergence of the lamina and the mechanics of its unfurling. Suffice it to state here that the unrolling is effected by the growth of the cells of the upper water tissue lying above and beside the principal veins, and along either side of the midrib. By the activity of the former cells the principal

veins are pushed above the general surface of the lamina-halves, and these, which up to this time have been perfectly smooth, acquire their characteristic ribbed appearance. Without the formation of these ribs, the broad, thin lamina-halves would lack the strength to hold themselves in a horizontal position. Thus we have the formation of a device essential to the proper functioning of the leaf postponed to the last possible moment, a fact in harmony with the relatively late appearance of the lamina-halves themselves. These statements will acquire greater significance when, in a later paragraph, we come to compare the banana with the palms.

The lamina-halves have been intercalated between the midrib and the marginal bundle. At the apex of the leaf, where the marginal bundles join with those of the midrib to form the vascular supply of the precursory appendage, we may well expect difficulty in adjustment if the lamina becomes broad and blunt in this region, as it does in the large, mature leaves of all varieties of *Musa sapientum* with which I am familiar. The left or outer half of the lamina is rolled about a rod, so to speak, and since it is closely pressed by the sheath of the next older leaf which encloses it, it is of mechanical necessity rolled flat. The marginal bundle has been forced to accommodate the growth of the blade. In consequence of this, this side experiences no difficulty in unfurling. On the right side, however, the lamina grows into a free space prepared for it by the enlargement of the midrib. Even here, it is true, there is not much wasted room, but the folds are not so tightly packed as on the opposite side. Here the marginal bundle does not keep pace with the growth of the apical portion of the lamina-half, which as a result becomes hooded or invaginated. When the pressure of the unfurling leaf loosens the coils of this lamina-half, forming a hollow cylinder, this hood enlarges to become a dome closing off the coils (see Skutch, 18, fig. 11). But the tissues are now too mature to yield further, and the entire cap is torn off as the leaf finally spreads out. Thus the right half of the lamina is terminated by a torn margin, in large leaves 12 cm. across. Some of the strain of mechanical adjustment at the apex may be inferred from the fact that the veins, as they are about to join the marginal bundle, are on both lamina-halves turned sharply back through about 140°. Then they apply themselves to the inner face of this bundle and run up into the precursory appendage.

Whether or not the apex of the lamina is torn in unfolding depends entirely on its size and shape. In the narrow leaves of sword-suckers, and even in the smaller of the broad leaves subsequently produced by these, the right side is able to flatten out without becoming lacerated.

Meanwhile the hyaline wing, now often tinged with red, has expanded until in large leaves it is 2.5 mm. or more wide. The fact that it never develops chlorophyll also suggests that this tissue is quite distinct from the lamina-half proper. The first sunny day suffices to shrivel it as far as the marginal bundle. In the abacá or Manila hemp plant (*Musa textilis*)

the marginal wing on the left side usually adheres so strongly to the surface of the coil lying adjacent to it in vernation that, when the leaf unfurls, it pulls away from the marginal bundle and remains attached to the lower surface of the lamina, as a straight, narrow, brown line, running almost parallel to the left margin. Although not every leaf of the abacá shows this mark (its failure to appear is perhaps a result of the atmospheric conditions prevailing at the time of the emergence and unfurling of the leaf), I do not remember a single large plant which failed to show it on at least some of its leaves; so, if the plant as a whole is considered, it is a good specific character. At the same time, the precursory appendage, which varies a good deal in texture in various species of *Musa*, shrivels up and falls away, if indeed it was not already torn off along with the dome on the apex of the right lamina-half.

Another late development in the life of the lamina is the enlargement of the cells along the lower side of the pulvinar band to form the motor tissue which allows the lamina-halves to fold downward beneath the midrib in the middle of a dry day, and raises them again in the afternoon. The final act in the adjustment of the leaf is the tearing of the lamina-halves into narrow strips by the wind. I am almost inclined to consider this a belated act in its development since, although it is a perfectly passive laceration, the leaf is not entirely adjusted to its environment on the plantation until it becomes, in this manner, falsely pinnate. In the abacá, the first tears across the lamina-halves are actually made during the emergence of the leaf from the top of the false-stem. Very soon after the appearance of any particular length of the lamina, it begins to unroll. The portions immediately basal to it are still held within the false-stem, and of course are not able to follow, but the forces acting to bring about the unfurling of the outer lamina-half are sufficiently strong to create transverse tears reaching to the midrib, and these permit the expansion to proceed by steps.

Every banana leaf I have ever examined closely was rolled in the same way, the right half covered by the left. This constant form of vernation is associated with the invariable direction of the spiral of phyllotaxy, which rises from the right to the left of an observer facing the stem. These statements are true not only of the numerous varieties of *Musa sapientum*, but also of *M. rosacea*, *M. malaccensis*, *M. tomentosa*, *M. sanguinea*, and *M. textilis*, which constitute all of the species I have been able to examine. The direction of development is a character which is constant for whole species, possibly even for the whole genus *Musa*.

AN INTERPRETATION OF THE DEVELOPMENT OF THE LAMINA IN THE LIGHT OF THE PHYLLODE THEORY

In attempting an interpretation of the morphology of the leaf of the banana, we are faced with a two-fold problem. In the first place, it is necessary to define the relation of the lamina-halves to the remainder of

the leaf. Secondly, it is desirable to determine the morphological value of the midrib itself, whether we are to regard it as the equivalent of the sheath, petiole, or midrib of the normal dicotyledonous leaf. Since the development of the lamina proceeds in two distinct steps, (1) the formation of the midrib and (2) the outgrowth of the lamina-halves from it, the two questions which we have proposed are in a measure independent of each other. The first, which seems to me the more important, is fortunately the easier of solution, and the validity of our conclusions will not be seriously affected by the morphological value we ascribe to the midrib.

A considerable amount of evidence points to the conclusion that the lamina-halves of the banana are a secondary development, and not homologous with the expanded portion of dicotyledonous leaves. A comparative view of the thin, expanded portion of the lamina, variously called the "wing" or "blade," as distinct from the rhachis or midrib, in different stages in the series of vascular plants, lends weight to this view. In the Hymenophyllaceae, Polypodiaceae, and other leptosporangiate ferns, the wing is formed on the sides of the rhachis by the activity of a series of marginal cells or initials, which are formed, in some cases at least, from the very earliest segments of the terminal growing point of the frond (Bower, 5, Pl. XXI, fig. 30). In the more bulky fronds of the Osmundaceae and Marattiaceae, definite marginal cells can not be recognized, although the wings are still formed on the sides of the originally terete rhachis by marginal growth. In the leaves of many dicotyledons, the future midrib (or rhachis) is the first portion to develop, and the wings first appear as paired outgrowths from its sides. This circumstance was already known to Trécul (19) who did not, however, devote much attention to the point. It is evident from his descriptions and figures that this is the mode of origin of the wings of *Liriodendron* (Pl. 21, fig. 47), *Citrus* (Pl. 22, fig. 24), and *Rumex* (Pl. 25, fig. 168), but in other cases their development in this manner is not so clear. Mrs. Arber (3) found that the wings of the lamina of a number of species which she studied develop as marginal outgrowths from the future midrib. In *Trifolium* the wings of the leaflets arise in much the same way from the ventral faces of their respective midribs. In *Syringa*, however, "the petiole passes almost insensibly into the lamina." Foster (8) observed that the wings of the leaflets of *Aesculus* originate from meristematic ridges along the ventral faces of their future midribs, much as Mrs. Arber described for *Trifolium*.

For a description of the exact manner in which this outgrowth of the wings occurs, and the meristematic layers involved, we must turn to the researches of Noack (15) and Krumbholz (13) (see also Schüepp, 17). The former found that the wings of the leaf of *Pelargonium* are produced by a marginal meristem in which the histogenetic layers behave very much as in the terminal growing point of the leaf, or even in the growing point of the stem. The hypodermal layer of the meristem is responsible for the origin

of all of the mesophyll, while the epidermis is formed by an independent dermatogen. In *Oenothera*, according to Krumbholz, the rôles played by the various meristematic layers in the formation of the wing are somewhat different but (and this is what concerns us in particular) the expansion of the wing is the result of a process which very much resembles apical growth.

Mrs. Arber (3) has already called attention to the fact that the wings of a number of monocotyledonous leaves, including *Sagittaria*, *Aponogeton*, and *Potamogeton* in the Helobiae, *Calla* and *Arum* in the Araceae, and *Smilax* in the Liliaceae, originate as lateral outgrowths of the midrib. In so far as the primary fact of the formation of its lamina by the "winging" of its midrib is concerned, these monocotyledons essentially resemble numerous dicotyledons, but in certain of them an additional process, not known to occur in the latter group, plays a subordinate part in the development of the lamina. This process is invagination, which Mrs. Arber has demonstrated to be of major or subordinate importance in the development of a large number of monocotyledonous leaves, and considers one of the mainstays of the phyllode theory.

The banana agrees with the ferns, a large number of dicotyledons, and many other monocotyledons in the formation of the lamina by the "winging" of the rhachis or midrib, nor is it distinguished from them by the participation of invagination in its development. But the point of origin of the meristem from which the wing is developed is different (so far as I am aware) in *Musa*, and other Scitamineae, from all other plants, with the single exception of the palms. In ferns and dicotyledons, the wings originate at an early age before the marginal tissues of the midrib (or rhachis) have lost their meristematic character; in ferns from cells which from the very first are specialized for this function. But in *Musa* the lamina-halves do not commence to develop until a comparatively late date, when the marginal cells of the midrib have lost their meristematic nature, and have differentiated into a scarious border; hence their initiation is relegated to deeper, less specialized tissues, and the margin of the developing lamina-half is occupied by a hyaline wing, instead of by the formative meristem itself, as in *Pelargonium*. It is for this reason, among others, that I consider the lamina-halves of the banana a secondary development, and in no sense homologous with those of the dicotyledonous leaf.

The production of marginal outgrowths is not restricted to that portion of the leaf which is morphologically lamina, but may occur in manifold forms in organs of different morphological value. Foster (8) has described the broadening of the inner bud scales of *Aesculus*, which he has demonstrated to be developments of the leaf-base, by wing-like outgrowths from their sides (l.c. Pl. L, fig. 30). This production of a pseudo-lamina by a leaf-base in a dicotyledon is of particular interest, in view of the theory which I shall presently advance, that the leaf of the banana bears just such a pseudo-

lamina. The venation of the wings of the bud scale of the horse-chestnut is irregularly dichotomous, not parallel as in monocotyledons, which should indicate that the form of venation alone is of little value as an indication of the homology of the lamina.

As the result of the activity of two entirely independent meristems, one lamina-half of the banana frequently extends farther down the petiole than the other. In certain varieties of *Musa textilis* the right half usually



TEXT FIG. 19. Base of the lamina of a leaf of *Musa textilis*, showing the unequal basal extension of the two halves of the lamina.

extends far below the left, and in one case an inequality of 17 cm. was measured (text fig. 19). There is no general rule as to which side is produced the farthest basally. In *Musa tomentosa* and *M. sapientum* variety Gros Michel, for example, the left side extends below the right, while in *M. sanguinea* and many varieties of *M. sapientum* the two lamina-halves meet

the petiole at approximately the same level. The nature of this basal inequality is entirely different from the obliquity or basal asymmetry of many dicotyledonous leaves, which is caused merely by the unequal outgrowth of the basal lobes, and these may nevertheless meet the petiole at the same level. In some species of dicotyledons whose leaves possess a distinct, marginless petiole, it is possible to find numerous examples in which the lamina-halves meet the petiole at different levels, just as in *Musa textilis*, but from the smaller size of the laminae the inequality is much less conspicuous. *Cornus florida* and *Quercus palustris* furnish many leaves of this character. In one case as in the other, the basal inequality of the lamina-halves is lasting testimony of the fact that they are of independent origin.

The very late appearance of the lamina-halves, compared with the other portions of the leaf, also points to a phylogenetically later origin. It is almost a truism in embryology that the characters or organs more recently acquired by the ancestral line make their appearance in the developing individual at a relatively late date; the ontogeny repeats the phylogeny. While it must be admitted that ecological requirements sometimes obscure this law, its general validity is supported by the vast majority of cases. The delayed appearance of the lamina-halves, while perfectly evident from text figures 4-11 and the description which accompanies them, is further supported by measurements of leaf rudiments in various stages. Table 1 gives the dimensions of successive leaves of the same plant. Reading the table from below upward, it is seen that the lamina-halves do not appear until the future lamina (that is, the midrib which will support it) exceeds 1.1 cm. in length. In the next older rudiment, the lamina half on the left side (the only one it is possible to measure with accuracy in very young rudiments) is only one forty-second the length of the lamina. If I have erred in determining the breadth of the lamina-half at this stage, it is on the side of generosity, for in the succeeding rudiment the ratio of breadth to length is 1 to 66, whence there is a regular increase until the mature proportions of 1 to 5 or 6 are attained (cf. also text figs. 14-17). In every case, the lamina-half was measured at its broadest point.

In dicotyledonous leaves the petiole is a relatively late development, intercalated between the lamina and leaf base after the former is well advanced (Trécul, 19; Deinema, 6). In the banana the condition is just the reverse. The petiole is easily distinguishable in rudiments where the lamina-halves are in their earliest stages. Because of the gradual transition from the sheath to the petiole (see Skutch, 18, p. 361) it is not possible to place the limit between them in very young rudiments with sufficient accuracy, and it was found desirable to measure them together in making table 1, but none-the-less, a distinct petiolar portion may be discerned at a very early stage, and in cross-sections it is easy to distinguish it from the sheath by the arrangement of the longitudinal septa.

The length of the future midrib is to a certain extent determined before

the lamina-halves are initiated, or at least while they are still quite young, and their outgrowth from the sides of the former is more or less independent of its size. An interesting result of this is the great variation in the *proportions* of the various leaves produced during the course of the life of a single plant. The first leaves formed by a young sucker are of the "sword" type, long and narrow, practically linear in outline. As the sucker matures, the relative breadth of the lamina increases until the oblong leaves of the mature type are formed. The change in the proportions of the leaves is quite as striking as their change in absolute size. If one cuts between a young sucker and the parent rhizome with a sharp machete, thereby severing the organic connection between the two without destroying the root system of the former, the leaves of the former which unfold after the operation, while increasing considerably in length, show a very much greater increase in breadth, showing that the two formative processes have been influenced to different degrees.

The evidence presented seems to favor the conclusion that the lamina-halves, the essential assimilating portion of the leaf, are a secondary development, acquired late in the history of the species and in no wise homologous with the lamina of a dicotyledonous plant. This circumstance points to the fact either that the remote ancestors of the present-day bananas were devoid of true laminae, or that they lost them at the same time that these substitute laminae, or phyllodes, were acquired. Our knowledge of the phylogeny of the Musaceae is too slight even to hazard a conjecture as to the exact mode by which the present type of leaf was developed.

The second point which we are called upon to settle is the morphological nature of the midrib. Mrs. Arber (1) has pointed out that the more or less radial arrangement of the vascular bundles in the precursory appendage strongly resembles the disposition of the bundles in the petiole of many dicotyledonous leaves. She has compared this appendage to the corset of the sepals of *Clematis*, *Passiflora incarnata*, and certain other dicotyledonous flowers. These sepals are developments of the leaf-base or sheath, while the corset is the vestige of the petiole. Many bud scales, too, as in *Fatsia japonica*, are modified leaf bases, and are surmounted by a small appendage which shows a radial disposition of the vascular bundles and represents the reduced petiole. Hence, she considers the precursory appendage in the banana to be the vestige of the tip of the petiole, while the lamina is a pseudo-lamina formed by the expansion of the distal portion of the petiole, but not involving the very apex.

The effort to explain the precursory appendage in the banana on morphological rather than on physiological grounds (cf. Genter, 9) seems a step in the proper direction. The purely mechanical function of the appendage in forming a passage for the portions of the leaf which follow it up the compact false-stem is obvious, but although this may account for the retention, or even the exaggeration, of a structure already present, it does not suffice to

explain its origin. I regard its physiological importance as negligible, for leaves with the appendage cut off as soon as it appears from the false-stem developed normally and grew as rapidly as others on which it was allowed to remain until it withered, which was usually long before the leaf had completely emerged.

But while I am entirely in accord with Mrs. Arber in regarding the appendage as a vestige of the petiole, and the blade as a pseudo-lamina or phyllode, I cannot join her in considering the appendage as the vestige of merely the apical extremity of the petiole, or the lamina the expansion of its distal portion. Neither the (apparent) petiole nor the midrib of the banana leaf shows any of those anatomical characters from which Mrs. Arber deduces the petiolar nature of the precursory appendage, or of the leaves of the Iridaceae and many other monocotyledons. The bundles are all normal in their orientation. All of the changes which occur in passing from the sheath to the petiole, which in its distal portion presents almost exactly the same cross-section as the greater portion of the midrib, show clearly that the development of the abaxial side has been favored at the expense of the adaxial, and this has resulted in pushing back the central lacunae from the upper surface (cf. Skutch 18, figs. 23 and 24). The process is similar to that by which Goebel (10, p. 278) explains the origin of the unifacial leaves of species of *Iris* and *Allium*, etc. It is true that the same process, carried a step farther, would account for the structure of the precursory appendage itself, but it would not explain the presence of this in the first place.

It seems most logical, therefore, to consider the entire leaf, upward to the base of the precursory appendage, as morphologically leaf-sheath. Another mode of viewing the problem leads to the same conclusion. The almost longitudinal veins converging at the apex, characteristic of the entire surface of the laminae of very early seedling leaves (text fig. 2) are found only nearer and nearer the apex in progressively later leaves. In leaves of the fully mature type, such an approximately parallel and longitudinal arrangement of bundles is found only in the base of the appendage, before it becomes a solid organ (cf. text fig. 6). It seems, then, that the process by which the lamina-halves are formed encroaches lower and lower in the leaf rudiment, while that portion which represents the seedling leaf is represented only at the very apex of the lamina of the mature leaf. The seedling leaf itself is of the liliaceous type, which Mrs. Arber (4) considers to be a leaf-base phyllode.

The complex history of the development of the leaves of palms has induced many to follow its course. The view of Naumann that the layers of the plicate leaf arise by the splitting of an originally solid rudiment has been effectively refuted by Hirmer (12), who is in accord with all other of the more recent investigators (6, 7), that they arise by the folding, or more properly (2), the invagination, of the lamina. The young rudiment of the leaf of a feather palm shows, in addition to the basal sheath, a comparatively very massive rhachis, bordered by a relatively narrow and in-

significant marginal tissue which is the rudiment of all the pinnae. This marginal tissue soon begins to form the folds so characteristic of the palm leaf. The invagination or folding of the tissue does not, however, extend to the very edge, but is restricted to an at first narrow zone, while a peripheral band remains unfolded. This binds together the tips of the pinnae and is torn away from them when the leaf finally expands. Only in *Chamaedorea*, among all the species which have been investigated, do the folds extend practically to the edge of the rudiment, and an extremely narrow border, which soon disappears, is left over.

In the production of a very evident rhachis before there is any trace of a lamina, the presence of a marginal wing which does not constitute a portion of the mature leaf, and the intercalation of the future lamina between the rhachis and the wing, the palm agrees closely with the banana. While I have not been able to find any definite statement that the unfolded margin of the palm leaf is older than the portion which forms the folds, I infer, from a statement by Hirmer that the cells of the former have early lost their meristematic character, that this is the case. In referring to the folded portion, I have used the word lamina advisedly, for the pinnae of a feather palm leaf constitute merely a split-up lamina, its divisions separated by the intercalary growth of the midrib, which thereby becomes a rhachis. If we were to imagine the lamina-halves of the rudiment shown in text figure 14 to grow more rapidly in a longitudinal direction than the midrib, they must inevitably be thrown into folds, since the wing could not, and the midrib does not, keep pace with them. If these folds were then to become separated by the death of the tissue at the upper or lower creases, we would have something like a feather palm.

I have restricted my discussion to the feather palms because of their closer resemblances to the banana leaf, but it would apply to the fan palms equally well for, according to Eichler, the only fundamental difference between the two types is the originally shorter rhachis of the latter, and its failure subsequently to elongate.

The two largest and most striking types of monocotyledonous leaves, exemplified by the *Palmae* and *Musaceae*, are not so fundamentally different in their mode of development as would appear at first sight, great as the differences undoubtedly are. One further point seems worthy of mention, and that is the much earlier development of the lamina in the palms. Thus in a rudiment of *Phoenix spinosa* only 0.5 cm. long, the process of disorganization of the upper creases, which results in the separation of the pinnae, had already begun (7). In a leaf-rudiment of the banana of the same size, the lamina has not yet begun to form, and when the leaf emerges, the lamina is still entire. As a result of the very early separation of the pinnae of the palms, while their tissues are still embryonic, they are in some cases (*Cocos*, *Chamaerops*) able to regenerate an epidermis to close off the wound, but the splitting of the banana leaf into "false-pinnae" is accom-

plished by outside agencies after the leaf is mature, and the wound is closed merely by the suberization of those cells which happen to border it, as is the case in many palms. An attempt to throw the banana leaf into the folds necessary for its mechanical support, resulting in the formation of ribs, occurs at the time of unfurling, instead of very early in the bud. The palms certainly possess a more highly developed type of leaf than the Musaceae, despite the fact that taxonomic works generally place them lower in the scale.

SUMMARY

1. In the rudiment of the banana leaf the lamina-halves arise at a relatively very late period. In the future sheath, petiole, midrib, and precursory appendage, the lacunar regions are clearly differentiated from the outer walls and septa, and in these the principal vascular bundles are laid down, before the lamina-halves appear.

2. The lamina-halves arise from the edges of the midrib, but interior to the hyaline margin and the outermost vascular bundle of each side of the former. This margin, which early loses its meristematic condition, becomes the evanescent membranaceous wing which borders the newly unfurled lamina, and the vascular bundle becomes the marginal bundle of the lamina.

3. The lamina-halves differentiate in a centripetal and basipetal direction. The apex of each half precedes the base in differentiation, and the marginal region that near the midrib.

4. The right and left halves of the lamina originate at the same time, but entirely independently of each other. In some species of *Musa*, one half may extend down the petiole many centimeters beyond the other.

5. The varying proportions of the leaves indicate that the outgrowth of the lamina-halves is in a measure independent of the length of the midrib, and is determined at a later period than this.

6. The lamina of the banana leaf is a secondary development, and its morphological value is entirely different from that of typical dicotyledonous leaves. Its midrib is morphologically a portion of the leaf-base or sheath, while the precursory appendage is probably a vestigial petiole.

7. The present study is in accord with the phyllode theory of the monocotyledonous leaf as propounded by Mrs. Arber.

8. In the early development of the midrib or rhachis, and the intercalation of the lamina between this and a marginal wing which does not form a portion of the mature lamina, the banana shows points of agreement with published descriptions of the origin of palm leaves.

In conclusion, I wish to express my deepest gratitude to Professor Duncan S. Johnson for his warm interest and his continued support of my tropical work; to Dr. John R. Johnston, Director of Agricultural Research of the United Fruit Company of Boston, for allowing me the use of the

Company's research stations in Central America, and for many other courtesies extended to me by the Company; and to Mr. Joseph H. Permar, director of the station at Almirante, Panama, where the present study was completed, for his unfailing coöperation during my sojourn there.

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GROWTH AND NITROGEN METABOLISM OF SQUASH SEEDLINGS¹ I. VARIATIONS AT DIFFERENT SEASONS OF THE YEAR

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Seasonal peculiarities in the development of plants grown at similar temperatures are a consequence of variations in the duration, intensity, and quality of light, the differences reaching a maximum at the time of the summer and winter solstices.

The effect of light in modifying plant processes has been brought into prominence in recent years particularly through publications of Garner and Allard (1, 2, 3) on the influence of the duration of light upon plant growth and sexual reproduction and by the researches of Kraus and Kraybill (6) which show the importance of carbohydrates for growth and sexual reproduction. The trend of the investigations to be reported herein has been in relation to the influence of the amount of newly synthesized carbohydrates on growth and the utilization of nitrogen. Consequently, those light factors which influence carbohydrate synthesis and utilization are chiefly to be considered. Possibly the three light factors—intensity, duration, and quality—have all played a rôle in causing the observed results but undoubtedly differences in intensity and duration at different seasons of the year have been of relatively greater importance than differences in quality.

Since one of the most characteristic differences in the growth and metabolism of squash seedlings at different times of year has been in connection with the developmental history of the chloroplasts, particular attention will be paid to describing the results pertaining thereto and to discussing these phases of the problem.

Zacharias (14) noted that the chloroplasts are the parts of the leaf cell richest in protein. Molisch (8) confirmed this observation. He studied the distribution of proteins in leaves by means of the xanthoproteic, biuret, and Millon's tests, after removal of the green color. He found that green leaves gave intense and yellow leaves very weak color reactions. Microscopic examination revealed that the stroma of the chloroplasts disappears during yellowing. Variations in the protein content of green leaves were found difficult to detect by the macroscopic reactions because the chloroplast stroma contains so much protein that positive results are always

¹ These investigations were conducted during 1926-8 in the Department of Physiological Chemistry of Yale University where the writer held a Sterling Research Fellowship.

obtained. He observed that the nucleole and other nuclear material participate only a little in the xanthoproteic color reaction of leaf tissue.

Meyer (7) agreed with Molisch that it is difficult to detect differences in protein content of healthy green leaves of the same age by means of the xanthoproteic reaction but he found that there are marked differences in the intensity of the color reaction in leaves of different ages due to variations in the amount of "ergastic" protein in the chloroplasts. In leaves of *Tropaeolum majus* he observed that there is a breaking down of the reserve proteins with age. The plastids become smaller and the chlorophyll content decreases. The chlorophyll entirely vanishes from the leaf with the complete disappearance of the ergastic proteins from the plastids. The nucleus changes much less in volume and the nucleoles do not become noticeably smaller as long as some chlorophyll is present in the chloroplasts. If light is excluded from the leaf surface for several days, the xanthoproteic reaction produced by the chloroplasts becomes noticeably weaker. Both darkened and undarkened leaves yellow more slowly if they are on a plant whose growing stem tip has been removed. Detached leaves yellow more slowly and more irregularly in darkness than leaves left on the plant. He concluded that the yellowing is carried on independently by the leaf protoplasm and although the main axis may have an accelerating effect it does not have to participate in the process. He observed that the leaf often yellows much earlier than it would from causes of age and supposes that a weakening of the organism occurs. He suggests that we may seek the influence of age in the relatively smaller production of carbohydrates by older leaves (13). His experimental results, however, indicated that shortage of carbohydrates could not be the only factor involved in the ageing phenomena.

Ullrich (12) stated that not much of the protein of the chloroplasts is to be reckoned as a temporary food reserve although the stroma of the plastid contains some reserve protein. He showed that a close relation exists between the color of the leaf, its protein content, and the size of the chloroplasts.

A diminution in the plasma and chlorophyll content of chloroplasts similar to that attributed by Meyer to ageing processes may also be brought about relatively early in the life of a plant by environmental conditions which produce an accumulation of carbohydrates in connection with a much limited nitrogen supply. The color changes in leaves, and the changes both in the chlorophyll and the plasma content of the chloroplasts in the stems of tomato plants having available different proportions of carbohydrates and nitrogen, was followed by Kraus and Kraybill (6). A summary of their observations follows:

It was observed throughout the experiments that as the plants became less vegetative, the leaves began to lose their fresh green color, to turn grey-green, and finally yellowish. In spite of this fact, carbohydrate storage in the stems continued. In the very vegetative

stems, small plastids containing chlorophyll were to be found in the cortical cells and in the pith cells even to the center of the largest stems and especially toward the tips. When the available nitrogen in the soil was limited, either by drying out the soil or withholding nitrogenous fertilizers, the plants began to turn yellow. This was accompanied by a complete disappearance of the green pigment from the plastids within the cortical and pith cells, and apparently the disappearance of many of the plastids themselves, especially when deposition of starch grains within the cells became rapid. On supplying nitrate to the soil, such plants as were non-vegetative first began active growth at the stem tips. This was associated with the greening of the smaller, younger leaves and a very rapid disappearance of the starch grains from the pith cells of the stem, first near the tip, and then progressively down the stem to its very base. Plastids again began to appear in these cells and later took on a bright green color. These plastids were especially abundant in the cells of the newer growth produced after the application of nitrate fertilizer, but also occurred in the cells of the older growth.

EXPERIMENTAL METHODS

Seedlings of Hubbard squash (Large-Warted) have been used in these investigations. Seeds of uniform size were selected and placed to germinate between layers of moist filter paper in a loosely covered dish. When the radicles were 2 to 3 cm. long, the seed coats and green membranes were removed and the seedlings were planted in pulverized quartz (no. 3) which had been washed several times in distilled water. New sand was used for each experiment. The culture dishes consisted of two-quart, glazed earthenware jars of shallow type. An opening about one-half inch in diameter was broken through the bottom of each jar to furnish drainage. The jars were placed in shallow granite pans, designed to hold the surplus supply of nutrient solution.

Two types of nutrient solution were used, one containing nitrogen and the other lacking nitrogen in any form. Preliminary experiments were conducted using different nitrates such as the calcium, potassium, and ammonium salts and also various combinations of the three salts. It was found that after the seedlings had emerged above the sand and the cotyledons were exposed to the light, nitrogen in the form of ammonium nitrate produced about as rapid growth as any of the other forms or combinations of forms which were tested. Ammonium nitrate was considered a desirable compound to use in these investigations as its use did not involve the possible intake of a non-nitrogenous base in association with the nitrogen. The calcium, magnesium, and potassium contents of the solutions containing and lacking nitrogen could be made the same. The solutions used in most of the experiments were prepared according to the following formulas:

Solution containing nitrogen:

| | |
|------------------------------------|-----------------------|
| 0.2% magnesium sulfate | 0.1% calcium chlorid |
| 0.3% monobasic potassium phosphate | 0.15% calcium sulfate |
| 0.143% ammonium nitrate | |

Solution lacking nitrogen:

Same as above except that ammonium nitrate was omitted

Iron was present in the sand in sufficient amounts to meet the requirements of the seedlings. Traces of the other metals necessary for growth but not supplied in the nutrient solution must have been present in the seeds or sand, or in both, in quantities adequate to provide for the needs of the seedlings during the relatively short periods allowed for their growth. Evidences of deficiencies were not observed during the growth periods herein described. In a few experiments in which some of the seedlings were allowed to grow for somewhat longer periods, abnormalities developed in the nitrated plants which correspond in several respects with those described by other investigators in the case of tomato, tobacco, and certain legumes and said to be caused by lack of boron. The nutrient solutions usually were not applied until the second to third day after the seedlings were planted in the sand as it was found that ammonium nitrate tends to have a slightly retarding effect on the very early phases of growth of squash seedlings, before the cotyledons are exposed to the light and before carbohydrate synthesis has begun. Squash seedlings are capable of synthesizing carbohydrates very soon after the cotyledons are exposed to the light, which possibly partially accounts for the fact that they can use ammonium nitrate advantageously. The observation is in agreement with results obtained by Prianischnikow (9) in experiments in which ammonium nitrate was employed as a nutrient. He found that if carbohydrates were deficient, toxic effects resulted from the accumulation of the ammonium ions but if carbohydrates were present in adequate amounts toxic effects did not occur, since the ammonium ions were used in the synthesis of organic nitrogen compounds.

One hundred cc. of the nutrient solution were added to each culture in the first application and thereafter the same amount was given each day except during the later development of the cultures receiving nitrogen. When the latter plants were between two and three weeks old, 200 cc. of solution were added each day. In general, the solutions were supplied in amounts sufficient to maintain growth at the maximum rate for each type of nutritive condition. Previous to applying the fresh solutions, 150 cc. of distilled water were poured on the sand each day, care being taken to wet the entire surface of the sand. The water washed out much of the remaining nutrient solution and the excess drained through into the pans and was then poured out. Ammonia-free water was added at other times during the day in amounts sufficient to maintain a layer of solution in the pans from $\frac{1}{4}$ to $\frac{1}{2}$ inch deep.

The cultures were placed on a bench at the end of a greenhouse where they were not in close proximity to other plants and where it was possible to keep the cultures from being sprayed with tap water when other plants in the greenhouse were being watered. The bench on which the cultures were placed was covered with several layers of clean heavy wrapping paper in order to exclude contact with surfaces which might harbor algae or

other microörganisms. Because of these precautions it was possible to produce relatively clean cultures. No traces of algae were found on any of the cultures grown without nitrogen in the nutrient solution except a slight amount on the surface of the sand of the cultures grown during

TABLE I. *Seedlings Grown in Light and Harvested at Different Stages of Growth. Thirty-two Plants. Seeds Contained 327 Mg. N. April 24 to May 17, 1927*

| Age and Cultural Conditions of Seedlings | Organs of Seedlings | Green Weights gms. | Dry Weights gms. | Total Nitrogen mg. | Percent-age of Nitrogen in Dry Weight | Percent-age of Nitrogen in Green Weight | Percent-age of Moisture |
|--|---------------------|--------------------|------------------|--------------------|---------------------------------------|---|-------------------------|
| May 3 Seedlings grown in sand 9 days | Leaves..... | 4.50 | 0.466 | 26 | 5.66 | 0.587 | 89.6 |
| | Stems..... | 23.2 | 1.756 | 34 | 1.92 | 0.145 | 92.4 |
| | Roots..... | 58.8 | 2.699 | 101 | 3.73 | 0.171 | 95.4 |
| | Cotyledons. | 53.5 | 4.204 | 143 | 3.40 | 0.267 | 92.1 |
| | Total..... | 140.0 | 9.125 | 304 | | | |
| - NH ₄ NO ₃ | Loss of N.. | | | - 23 | | | |
| May 10 Seedlings grown in sand 16 days | Leaves..... | 16.2 | 2.282 | 72 | 3.14 | 0.438 | 85.9 |
| | Stems..... | 23.4 | 3.859 | 56 | 1.45 | 0.239 | 88.4 |
| | Roots..... | 76.7 | 3.246 | 80 | 2.45 | 0.103 | 95.7 |
| | Cotyledons. | 54.0 | 4.532 | 82 | 1.80 | 0.151 | 91.6 |
| | Total..... | 180.3 | 13.919 | 290 | | | |
| - NH ₄ NO ₃ | Loss of N.. | | | - 37 | | | |
| May 17 Seedlings grown in sand 23 days | Leaves..... | 27.0 | 3.892 | 92 | 2.37 | 0.341 | 85.6 |
| | Stems..... | 46.2 | 5.690 | 72 | 1.26 | 0.155 | 87.7 |
| | Roots..... | 77.2 | 3.491 | 80 | 2.29 | 0.103 | 95.5 |
| | Cotyledons. | 55.0 | 3.869 | 50 | 1.29 | 0.090 | 92.9 |
| | Total..... | 205.4 | 16.942 | 294 | | | |
| - NH ₄ NO ₃ | Loss of N.. | | | - 33 | | | |
| May 10 Seedlings grown in sand 16 days | Leaves..... | 101.3 | 10.304 | 532 | 5.16 | 0.524 | 89.8 |
| | Stems..... | 137.6 | 7.770 | 141 | 1.81 | 0.101 | 94.3 |
| | Roots..... | 126.1 | 5.382 | 211 | 3.92 | 0.180 | 95.7 |
| | Cotyledons. | 98.4 | 5.914 | 167 | 2.83 | 0.169 | 94.0 |
| | Total..... | 463.4 | 29.370 | 1051 | | | |
| + NH ₄ NO ₃ | Gain in N.. | | | + 724 | | | |
| May 17 Seedlings grown in sand 23 days | Leaves..... | 192.0 | 23.779 | 785 | 3.30 | 0.408 | 87.6 |
| | Stems..... | 269.4 | 19.828 | 285 | 1.44 | 0.106 | 92.6 |
| | Roots..... | 133.6 | 7.397 | 233 | 3.15 | 0.174 | 94.4 |
| | Cotyledons. | 97.6 | 5.494 | 90 | 1.63 | 0.091 | 94.3 |
| | Total..... | 692.6 | 56.498 | 1393 | | | |
| + NH ₄ NO ₃ | Gain in N.. | | | + 1066 | | | |

November and December when the light conditions especially favored the development of algae. The cultures receiving nitrates usually had some algae growing on the surface of the sand after a period of about three weeks.

In the application of distilled water and nutrient solutions, particularly in the case of the cultures grown in the solution lacking nitrogen, extreme care was taken not to touch or wet the surfaces of the leaves. Wetting of the leaves, especially as they began to show signs of ageing, was to be avoided, since there was some possibility that losses of nitrogen from the

TABLE 2. *Seedlings Grown in Light and Harvested at Different Stages of Growth. Thirty-two Plants. Seeds Contained 327 Mg. N. June 2-17, 1927*

| Age and Cultural Conditions of Seedlings | Organs of Seedlings | Green Weights gms. | Dry Weights gms. | Total Nitrogen mg. | Percent-age of Nitrogen in Dry Weight | Percent-age of Nitrogen in Green Weight | Percent-age of Moisture |
|--|---------------------|--------------------|------------------|--------------------|---------------------------------------|---|-------------------------|
| June 8 Seedlings grown in sand 6 days | Leaves..... | — | — | — | — | — | — |
| | Stems and buds..... | 11.55 | 0.989 | 34 | 3.42 | 0.292 | 91.4 |
| | Roots..... | 24.93 | 1.836 | 59 | 3.22 | 0.237 | 92.6 |
| | Cotyledons..... | 34.75 | 4.274 | 236 | 5.53 | 0.680 | 87.7 |
| | Total..... | 71.23 | 7.099 | 329 | | | |
| — NH_4NO_3 | Gain in N..... | | | + 2 | | | |
| June 17 Seedlings grown in sand 15 days | Leaves..... | 16.02 | 2.782 | 83 | 2.98 | 0.517 | 82.6 |
| | Stems..... | 31.26 | 3.603 | 53 | 1.47 | 0.165 | 88.4 |
| | Roots..... | 59.52 | 2.928 | 75 | 2.55 | 0.125 | 95.1 |
| | Cotyledons..... | 53.44 | 4.246 | 82 | 1.94 | 0.153 | 92.0 |
| | Total..... | 160.24 | 13.559 | 293 | | | |
| — NH_4NO_3 | Loss of N..... | | | — 34 | | | |
| June 17 Seedlings grown in sand 15 days | Leaves..... | 115.8 | 15.456 | 737 | 4.78 | 0.636 | 86.6 |
| | Stems..... | 127.7 | 7.411 | 176 | 2.37 | 0.137 | 94.2 |
| | Roots..... | 133.2 | 6.019 | 239 | 3.98 | 0.176 | 95.4 |
| | Cotyledons..... | 105.6 | 4.883 | 147 | 3.11 | 0.139 | 95.3 |
| | Total..... | 482.3 | 33.769 | 1299 | | | |
| + NH_4NO_3 | Gain in N..... | | | +972 | | | |

seedlings might occur. The surfaces of the leaves and cotyledons were examined several times a day for the presence of insects. Extreme precautions were taken to prevent any accidental gain or loss of nitrogen by the seedlings.

In these studies emphasis has been placed on the growth of seedlings without extra nitrogen, although in each experiment a few cultures of seedlings receiving additional nitrogen were grown. The aim has been chiefly to study the utilization of a definite amount of nitrogenous food reserves at different times of year. In order to make comparisons readily between results of different experiments the data shown in tables 1 to 5 have been calculated as closely as possible on the basis of equivalent amounts of reserve nitrogen.

Total nitrogen determinations were made by the Kjeldahl method modified to include the determination of nitrates (10). The material to be used in the determination was allowed to stand over night in the salicylic acid-sulfuric acid mixture. Digestions were conducted in 800-cc. Kjeldahl flasks and were continued for one to three hours after the solution became colorless. The accuracy of the method, particularly with respect to the

TABLE 3. *Seedlings Planted in Sand October 8, 1927, and Harvested at Different Stages of Growth. Thirty-five Plants. Seeds Contained 324 Mg. N*

| Age and Cultural Conditions of Seedlings | Organs of Seedlings | Green Weights gms. | Dry Weights gms. | Total Nitrogen mg. | Percent-age of Nitrogen in Dry Weight | Percent-age of Nitrogen in Green Weight | Percent-age of Moisture |
|--|---------------------|--------------------|------------------|--------------------|---------------------------------------|---|-------------------------|
| October 26 Seedlings grown in sand 18 days | Leaves..... | 17.40 | 2.450 | 98 | 4.03 | 0.570 | 85.8 |
| | Stems..... | 40.60 | 3.622 | 63 | 1.75 | 0.156 | 91.1 |
| | Roots..... | 38.85 | 1.746 | 50 | 2.88 | 0.129 | 95.5 |
| | Cotyledons.. | 55.65 | 3.780 | 95 | 2.50 | 0.169 | 93.2 |
| | Total..... | 152.50 | 11.598 | 306 | | | |
| - NH ₄ NO ₃ | Loss of N.. | | | - 18 | | | |
| November 4 Seedlings grown in sand 27 days | Leaves..... | 29.64 | 4.025 | 120 | 3.00 | 0.406 | 86.4 |
| | Stems..... | 55.30 | 5.915 | 76 | 1.28 | 0.136 | 89.3 |
| | Roots..... | 45.50 | 2.030 | 59 | 2.89 | 0.128 | 95.5 |
| | Cotyledons.. | 46.60 | 2.975 | 50 | 1.67 | 0.106 | 93.3 |
| | Total..... | 177.04 | 14.915 | 305 | | | |
| - NH ₄ NO ₃ | Loss of N.. | | | - 19 | | | |
| October 26 Seedlings grown in sand 18 days | Leaves..... | 63.70 | 6.860 | 430 | 7.50 | 0.846 | 90.9 |
| | Stems..... | 98.00 | 3.535 | 184 | 5.20 | 0.188 | 96.3 |
| | Roots..... | 54.95 | 2.124 | 112 | 5.26 | 0.203 | 96.1 |
| | Cotyledons.. | 93.10 | 4.795 | 263 | 5.48 | 0.281 | 94.8 |
| | Total..... | 309.75 | 17.314 | 989 | | | |
| + NH ₄ NO ₃ | Gain in N.. | | | + 665 | | | |
| November 4 Seedlings grown in sand 27 days | Leaves..... | 199.5 | 19.32 | 1194 | 6.18 | 0.597 | 90.3 |
| | Stems..... | 227.5 | 11.10 | 282 | 2.54 | 0.123 | 95.1 |
| | Roots..... | 97.3 | 4.445 | 212 | 4.76 | 0.217 | 95.4 |
| | Cotyledons.. | 73.5 | 3.920 | 104 | 2.68 | 0.159 | 94.7 |
| | Total..... | 597.8 | 38.785 | 1792 | | | |
| + NH ₄ NO ₃ | Gain in N.. | | | +1468 | | | |

standardization of the acid and alkali used in the determinations, was checked by determination of the nitrogen content of samples of pure tyrosine.² The analysis yielded the theoretical amounts of nitrogen. The fresh tissues of seedlings were prepared for analysis by drying to constant weight in a vacuum oven at 65° C.

²I wish to thank Dr. H. B. Vickery of the Connecticut Agricultural Experiment Station for the pure tyrosine used in making these determinations.

TABLE 4. *Seedlings Grown from November 15 to December 17, 1927. Thirty-four Plants. Seeds Contained 325 Mg. N*

| Cultural Conditions of Seedlings | Organs of Seedlings | Green Weights gms. | Dry Weights gms. | Total Nitrogen mg. | Percentage of Nitrogen in Dry Weight | Percentage of Nitrogen in Green Weight | Percentage of Moisture |
|-----------------------------------|---------------------|--------------------|------------------|--------------------|--------------------------------------|--|------------------------|
| Sand not extracted with acid | Leaves..... | 31.4 | 4.04 | 147 | 3.64 | 0.469 | 87.1 |
| | Stems..... | 54.0 | 5.07 | 69 | 1.37 | 0.129 | 90.6 |
| | Roots..... | 28.0 | 1.78 | 51 | 2.85 | 0.180 | 93.7 |
| | Cotyledons. | 36.0 | 2.34 | 51 | 2.17 | 0.140 | 93.5 |
| | Total..... | 149.4 | 13.23 | 318 | | | |
| — NH ₄ NO ₃ | Loss of N... | | | — 7 | | | |
| Sand extracted with conc. HCl. | Leaves..... | 32.3 | 4.01 | 158 | 3.95 | 0.488 | 87.6 |
| | Stems..... | 52.5 | 4.89 | 67 | 1.37 | 0.128 | 90.6 |
| | Roots..... | 28.3 | 1.38 | 41 | 2.95 | 0.144 | 95.1 |
| | Cotyledons. | 35.9 | 2.58 | 49 | 1.88 | 0.135 | 92.8 |
| | Total..... | 149.0 | 12.86 | 315 | | | |
| — NH ₄ NO ₃ | Loss of N... | | | — 10 | | | |
| Sand not extracted with acid | Leaves..... | 122 | 13.24 | 723 | 5.49 | 0.593 | 89.2 |
| | Stems..... | 152 | 6.76 | 190 | 2.82 | 0.125 | 95.5 |
| | Roots..... | 57.7 | 2.72 | 126 | 4.70 | 0.221 | 95.5 |
| | Cotyledons. | 67.6 | 3.08 | 102 | 3.28 | 0.150 | 95.4 |
| | Total..... | 399.3 | 25.80 | 1141 | | | |
| + NH ₄ NO ₃ | Gain in N... | | | +816 | | | |
| Sand extracted with conc. HCl. | Leaves..... | 102 | 10.73 | 611 | 5.74 | 0.603 | 89.3 |
| | Stems..... | 108 | 6.59 | 156 | 2.29 | 0.140 | 93.8 |
| | Roots..... | 37 | 2.06 | 95 | 4.62 | 0.252 | 94.5 |
| | Cotyledons. | 61 | 3.05 | 100 | 3.28 | 0.164 | 94.9 |
| | Total..... | 308 | 22.43 | 962 | | | |
| + NH ₄ NO ₃ | Gain in N... | | | +637 | | | |

TABLE 5. *Total Nitrogen (mg.) Distribution in Different Organs of Seedlings, Seedlings in Series Shown at Left Having High and Those at Right Low Carbohydrate Synthesis*

| Time of Year | 1927 April 24— May 17 | 1928 June 8— July 6 | 1928 Oct. 10— 31 + 1% CO ₂ | 1928 Oct. 10— Oct. 31 | 1927 Oct. 8— Nov. 4 | 1928 June 8— July 6 Shaded 7-hr. Day | 1928 Nov. 15— Dec. 17 | 1928 Oct. 10— Oct. 31 Very Low CO ₂ |
|-----------------------------------|-----------------------------|---------------------------|--|-----------------------------|---------------------------|--|-----------------------------|--|
| Leaves..... | 92 | 93 | 96 | 108 | 120 | 129 | 147 | 150 |
| Stems + petioles.... | 72 | 63 | 104 | 86 | 76 | 68 | 69 | 64 |
| Roots..... | 80 | 80 | 67 | 67 | 59 | 49 | 51 | 29 |
| Cotyledons—unused residue..... | 50 | 36 | 50 | 44 | 50 | 38 | 51 | 78 |
| Total..... | 294 | 272 | 317 | 305 | 305 | 284 | 318 | 321 |

The squash seed contains relatively large amounts of nitrogen (6.15 percent) and fat. The nitrogen is stored chiefly in the form of globulins. The nitrogen content of three lots of thirty-two seeds each was determined. The seeds were soaked for half an hour in distilled water and the seed coats were then carefully removed. The embryos of each of the three groups of seeds were shredded into small pieces with a scalpel and the pulverized material was then dried to constant weight. Two samples of approximately 0.3 gram each from each lot of seeds were placed in weighing bottles and dried to constant weight after which Kjeldahl analyses were made. The following results were obtained:

| Weight of Seeds | I 5.338 gms. | II 5.304 gms. | III 5.355 gms. |
|-----------------------------------|-----------------|------------------|-------------------|
| Percentage of N in duplicate..... | 1-6.15 | 1-6.22 | 1-6.09 |
| Samples of the dry material..... | 2-6.11 | 2-6.32 | 2-6.16 |
| Total nitrogen in seeds..... | 327 mg. | 333 mg. | 328 mg. |

Difference between highest and lowest amounts found in seeds—6 mg.

Possible difference between different lots of seeds—not more than 15 mg.

RESULTS

Experiment 1. April 24 to May 17, 1927

Different lots of seedlings were harvested at the end of 9-, 17-, 24-, and 32-day periods of growth. Temperature and light conditions favored rapid development. Brilliant sunshine prevailed throughout most of the days during which the seedlings were growing. The temperature in the greenhouse at night was approximately 21° C. and during the day it varied between 25° and 35° C. There were 32 seedlings in each lot, for each one of the four dates of harvest. Two experiments were conducted simultaneously; in one, a definite quantity of iron in the form of ferric citrate was added to the nutrient solution and to the other no iron was given. The growth of the two groups of seedlings appeared to be so nearly alike that the results only of the experiment with seedlings grown without added iron will be described. The green and dry weights, moisture, and nitrogen content calculated in percentage of green and dry weights and the total amounts of nitrogen in the different organs of 32 seedlings are shown in table 1.

A. Seedlings Grown Without Extra Nitrogen

On May 3, the date of the first harvest, the seedlings had produced one leaf each but it was not full grown. Many of them were still folded along the midrib. The cotyledons had attained full size, were intensely green, and appeared to have a bluish green tinge. By use of the iodine test made on sections of the cotyledons it was observed that they contained fairly large quantities of starch. The total amount of nitrogen contained in

32 seeds of the size used in the experiment was 327 mg., most of which was stored in the cotyledons. In the early stages of growth the chloroplasts of the cotyledons were large, well rounded in outline, and of a vivid green color. During the first nine days the nitrogen content of the cotyledons had decreased to 143 mg. The roots contained 101 mg. Much of the nitrogen which was mobilized during this early growth accumulated in the roots. The hypocotyls had attained full length at this time. The stems were very short.

On May 10, when the seedlings were 16 days old, the cotyledons had lost much of their greenness and the color had changed from bluish to yellowish green. They contained very large quantities of starch. Their nitrogen had decreased from 143 mg. found to be present on May 3 to 82 mg. on May 10 (table 1, column 5). The plasma content of the chloroplasts appeared to have diminished in conjunction with the loss in greenness. The first leaves had reached full size. They ranged from 3.2 to 4.1 cm. in length and were still very green. The second leaf had attained almost maximum size for these conditions of growth. The roots had grown longer and were much more branched than at the previous harvest. In spite of the very marked increase in size and weight of the roots from May 3 to 10, their total content of nitrogen had decreased from 101 mg. to 80 mg. Apparently some of the nitrogen which had accumulated in the roots during the early phases of germination had been translocated to other organs.

During the following week, May 10 to 17, the cotyledons lost most of their greenness and on May 17 they contained only about one-sixth of their original amount of nitrogen. The leaves also had become yellow-green in color. Third and fourth leaves had developed since the previous harvest but they remained very small. The upper surfaces of all of the leaves were shiny. The first or oldest leaves were rapidly losing chlorophyll and becoming more yellow. By microscopic examination of sections of these leaves it was observed that the chloroplasts had decreased in plasma as well as in chlorophyll content. The nitrogen contained in the stems increased with each successive period of growth but the stems did not gain much in length. The number of lateral roots appeared to increase continuously although the rate of root growth was less during the latter part of the experimental period. The nitrogen found in the roots did not increase between May 10 and 17. The stems, hypocotyls, and younger leaves contained very large quantities of starch. During the latter part of the growth period the starch content of the cotyledons decreased noticeably. On May 17, and more definitely a few days later, it was apparent that the plasma content of the chloroplasts of the cotyledons and also of the older leaves which were turning yellow had very definitely decreased. On May 25, another lot of seedlings was harvested but the processes of ageing and degeneration of the tissues had advanced so rapidly that it seemed inadvisable to use the tissues for quantitative determinations of nitrogen.

The data given in column 4 of table 1 show that with the exception of the cotyledons the dry weights of all tissues increased continuously throughout the experiment. In column 6 of the same table it is shown that the percentage of nitrogen of the dry weights of all tissues decreased continuously. Both of these changes resulted chiefly from the accumulation of carbohydrates, particularly starch, in the tissues. With a limited supply of an important constituent for growth and metabolism, such as nitrogen, carbohydrates accumulate (4, 5, 6). This accumulation proceeds rapidly in plants such as squash which are highly efficient in carbohydrate synthesis.

Chief characteristics of May plants grown without an external source of nitrogen are very large root systems in proportion to the size of the tops, short stems, small leaves which age rapidly, and high content of starch and reducing substances in the tissues. Plate XXI, figure 3, is an illustration of a plant grown similarly but in a different experiment.

B. Seedlings Which Received Extra Nitrogen

The tops of the plants grew very rapidly and became large but the root systems were small in proportion. At the end of the experimental period these plants had four to five well-developed leaves ranging from 9.5 to 12 cm. in length and stocky stems varying from 15 to 22 cm. in length. The roots were profusely branched but were relatively short.

The cotyledons grew to be larger and remained green for a considerably longer time and a decrease in nitrogen content occurred more slowly than in those of the un-nitrated plants. At the end of the experimental period they contained a residue of 90 mg. of nitrogen whereas those of the latter plants contained only 50 mg. Only traces of starch were found in them. The chloroplasts of the cotyledons appeared to retain their form and green color longer than those of the un-nitrated plants. Although carbohydrates must have been synthesized rapidly, they did not accumulate in large amounts in any of the tissues; growth and the synthesis of organic nitrogen compounds, and respiration, apparently had proceeded so rapidly that the utilization of carbohydrates kept pace with their synthesis.

There was no definite evidence of ageing of the leaves. When the oldest leaf of the un-nitrated plants had lost most of its greenness and the chloroplasts had markedly degenerated, the corresponding leaf of the nitrated cultures appeared to be in a fairly healthy condition. There may, however, have been somewhat less protein in these leaves than there had been at an earlier stage. Figure 4 shows the appearance of nitrated plants of another experiment but grown at the same season of the year and having the same general appearance as those just described.

Experiment 2. June 2-24, 1927

This experiment was a duplicate of experiment 1 both as to methods and experimental results. The quantitative data are given in table 2.

Due to an accident in the drying oven with the plant tissues obtained from the last harvest on June 24, the quantitative results from this material cannot be reported. However, the appearance of the seedlings in the later stages of development was like that of the corresponding stages of the May seedlings.

Experiment 3. October 8 to November 4, 1927

An experiment similar to experiment 1 was conducted during October, the results of which are shown in table 3. The characteristics of the plants were intermediate between those of the plants grown in May and December. No description of the plants is necessary.

Experiment 4. November 15 to December 17, 1927

Preliminary experiments conducted during the previous November and December (1926) had yielded plants with strikingly different characteristics from those grown during May and June. The root systems of the plants grown without additional nitrogen were much smaller but the tops much larger than those of similarly grown May and June plants. They presented a more vegetative appearance. The leaves were larger, usually more numerous, and remained green for a much longer time. The stems of these plants were several times longer than those of the May plants.

The seeds used in experiment 4 were placed in germinators November 9 and placed in sand November 15. The first application of nutrient solution was made on November 18. During the experimental period there were eleven days on which the sun shone almost continuously, eleven days that were cloudy throughout the day, and ten days that were partly cloudy. The limited number of hours of sunshine resulting from the short and frequently cloudy days and the low light intensity at this season of the year resulted in a very much smaller total amount of light than there had been during the May and June experiments. The approximate average temperature of the greenhouse at night was 21° C. and during the day 25° C., the latter being lower than the average day temperature of the May and June experiments.

The sand of four of the cultures of both the nitrated and un-nitrated series was digested in concentrated hydrochloric acid for five days and then washed free of acid. The object of this treatment was to remove the iron and also possible traces of other heavy metals which may have been in the sand and to observe the effect of this removal upon the type of growth. Thirty-two seedlings were grown in each series.

The quantitative results of the experiment are shown in table 4.

A. November–December Seedlings Grown Without Additional Nitrogen

Figure 1 shows the appearance of the plants. These seedlings like those grown during November and December the previous year were characterized by very large tops and small root systems in contrast with those of the May seedlings (Pl. XXI, fig. 3).

The cotyledons retained their greenness for a relatively long time but eventually the green became less and they appeared slightly greyish. As ageing progressed, the green disappeared abruptly first at the sides and later spreading inward. It had been observed that the cotyledons of the May seedlings turned yellowish green over the entire surface relatively early and thereafter the green became weaker and the yellow more noticeable. These differences in the color changes of the cotyledons of seedlings grown at opposite seasons of the year are undoubtedly an indication of differences in chemical processes occurring in connection with the mobilization of the reserves. At the time the experiment was terminated very few of the cotyledons were withered, many of them still having some green color. The cotyledons of one group of the thirty-two-day-old seedlings contained a residue of 51 mg. of nitrogen, those of the other group 49 mg. The fresh and dry weights of the cotyledons of the May seedlings were considerably greater than those of the December seedlings, the difference in dry weights being due chiefly to differences in carbohydrate content.

The leaves grew to be large in comparison with those of the May seedlings. The average length of the leaves from the first to the fifth nodes was 5.4, 3.8, 3.0, 2.3, and 1.0 cm. respectively. The third, fourth, and fifth leaves particularly were larger than the corresponding structures of the May seedlings. The average leaf area per plant was 60 sq. cm. Leaf area measurements of the May plants were not obtained but the average area per plant was certainly not more than half that of the December plants. The veins of the leaves were smaller, the blades were thinner, and their upper surfaces duller and less glossy than those of the May plants. Perhaps the most striking characteristic of the leaves was their vivid fresh greenness and the persistence of the green color. Even at the end of the thirty-two-day period of growth the first or oldest leaf of many of the seedlings was still green, although in some the green color had almost entirely disappeared. When the first leaf was yellowing the other leaves appeared still healthy and green whereas the second and third leaves of the May seedlings at the corresponding stage of development had a definite yellowish green color. Because of the shortness and frequent cloudiness of the days and the relatively low light intensity the accumulation of starch and reducing substances was much slower than it had been in the May plants. The chloroplasts in the leaves up to the time of yellowing were plump and very green.

The leaves contained 147 mg. of nitrogen in one series and 158 mg. in the other. Leaves of the October seedlings had contained 120 mg. of nitrogen and those of the May seedlings only 92 mg. The thinner leaves of the December plants had fewer cells and fewer chloroplasts per unit area of surface. If the leaf area of the May seedlings is estimated as one-half that of the December seedlings, it becomes evident that per unit of leaf surface the leaves of the May seedlings contained more nitrogen.

Slight differences in green weights of leaves grown at different seasons were found. The weights were highest in December and lowest in May, those of the October leaves being intermediate.

The stems of the December plants were somewhat longer than those of the October plants and much longer than those of the May plants and they contained less starch and reducing substances than those of the latter plants.

The roots of each of the two groups of seedlings weighed only 28 grams, those of the October plants weighed 45 grams and those of the May plants 77 grams. The amounts of nitrogen contained in the roots at different seasons were: December, group I, 51 mg.; group II, 41 mg.; October, 59 mg.; May, 80 mg.

The December plants had a higher content of nitrogen in the dry material of the leaves, roots, and cotyledons than had the May seedlings. There was not much difference in the total amount of nitrogen found in the stems at different seasons.

There was no noticeable difference in the appearance of the plants grown in the acid-treated or untreated sand. The similarity in weights of the different organs of the two series of plants may be observed in table 4. In experiments conducted during June there were marked differences between the seedlings grown in acid-treated and untreated sand. An account of the results will be reported in a later publication.

Table 5 summarizes the results obtained as to allocation of nitrogen in the organs of seedlings grown at different times of year. Data obtained in other experiments which will be described in a later publication are also presented. The latter experiments show the effect of high- and low-carbohydrate synthesis (conditions secured by varying the carbon dioxide content of the atmosphere) upon the distribution of nitrogen in the plant.

B. November-December Seedlings Grown With Additional Nitrogen

The most noticeable characteristics of these seedlings were their extremely vegetative condition, low carbohydrate content, and large proportion of tops to roots. In the early stages of growth the cotyledons had about the same color as those of the un-nitrated plants but later they became somewhat less intensely green than the latter. The green color persisted for a much longer time, however, than it did in the cotyledons of the un-nitrated plants. A residue of about 100 mg. of unused nitrogen remained in the cotyledons as compared with a residue of only 50 mg. in those of the latter plants.

The large, thin leaves were bright green and contained large chloroplasts. Their strengthening tissues were poorly developed. The blades had a dull surface and they wilted almost immediately after removal of the plants from the sand. The stems were slender and succulent, contained little starch and reducing material, and had relatively little development

of the strengthening tissues. The small size of the root systems and other characteristic features of appearance may be observed in figure 2.

There were large differences in green weights between the June and December plants but not so much difference in the absolute amounts of nitrogen contained in them. It appeared from the small amount of starch and reducing substances found in the December plants that carbohydrates might be a limiting factor in their growth.

There were differences between the plants grown in the acid-treated and untreated sand, those in the untreated sand being somewhat larger. About half of the plants grown in the acid-treated sand developed some mottled leaves, which had very brittle petioles and veins. The tips of the stems and very young leaves showed evidences of injury.

Losses of Nitrogen

Some of the foregoing tables have indicated unaccountable losses of nitrogen, particularly in the experiments conducted during May and June. Discrepancies in the nitrogen balance were as follows:

Experiment 1, April 24–May 14, 1927. In plants harvested after growing one, two, and three weeks, there was a deficiency of 23, 37, and 33 mg. of nitrogen, respectively.

Experiment 2, June 2–17, 1927. After a growing period of 6 days there was no appreciable change in nitrogen content but after 15 days there was a loss of 34 mg. of nitrogen.

Experiment 3, October 8–November 4, 1927. There were losses of 18 and 19 mg. in two successive harvests.

Experiment 4, November 15–December 17, 1927. In one experiment there was a loss of 7 mg. and in the other only 10 mg. at the end of a thirty-two-day growing period.

The losses during the November–December experiment were no greater than could be accounted for in the handling of the seedlings. The losses during the October experiment were somewhat greater but possibly were not significant. The losses during the May and June experiments cannot be accounted for.

DISCUSSION

On the basis of evidence obtained in previous experiments (11) it is supposed that the differences in development of roots of the May and December seedlings are a consequence of the seasonal variations in the proportions of carbohydrate and nitrogenous food substances which become available to the plants. The longer stems of the December plants in contrast with those of the May plants are an effect of the reduced light intensity and shorter period of illumination. Variations in light quality may possibly be partially responsible for the differences in stem lengths. Differences in thickness and size of leaves at opposite seasons of the year are also to be attributed to differences in light.

The changes in chlorophyll content and appearance of the chloroplasts of leaves of the un-nitrated May plants at successive stages of development seem to be somewhat like those described by Meyer as ageing phenomena. The details of the changes in structure of the chloroplasts of the squash seedlings have not yet been fully investigated. Examination of sections of fresh leaves and also of leaves killed with a fixative, embedded in paraffin, and stained have shown that rapid changes in the plasma as well as in the chlorophyll content of the chloroplasts occur relatively early if the nitrogen-limited leaves are developed during long days and under high illumination. Why should the phenomenon become evident earlier and more conspicuously in leaves exposed to higher illumination and to longer periods of illumination? It cannot be a consequence of lack of carbohydrates as postulated by Meyer in explanation of the changes in normally ageing leaves, since the leaves of the May plants contained more starch and reducing substances than those grown during December. It was found that with an abundance of nitrogen available to the June plants, the destructive changes either do not occur or they are much less noticeable. The question arises as to whether destructive processes take place more rapidly in the nitrogen-starved plants or whether they merely become evident earlier in consequence of limitation of repair materials. Evidence obtained in later experiments tends to show that the accumulation of carbohydrates, possibly especially of the more active forms such as the monoses, in proportion to the supply of readily available nitrogen results in destructive changes in the chloroplasts. Destructive processes may similarly occur in leaves of plants having a supply of available nitrogen but if so, the degenerative effects tend to be masked by the results of synthetic and restorative processes.

SUMMARY

A. Seedlings Grown Without an External Source of Nitrogen

1. Hubbard squash seedlings utilize their nitrogenous reserves in a somewhat different manner at opposite seasons of the year. Plants grown under the brilliant illumination of May and June and in days of normal length have short stems, large root systems, and relatively small, thick leaves. Plants similarly grown during November and December under light conditions typical in this latitude for this season of the year have much longer stems, smaller root systems, and large, thinner leaves, the third, fourth, and fifth leaves especially being much larger than the corresponding leaves of the May plants.

2. The formation of carbohydrates occurs much more rapidly in plants grown in June. Within a relatively short time cotyledons, stems, and leaves contain very large quantities of starch. Somewhat later large amounts of reducing substances accumulate.

3. The strengthening tissues are more developed in plants grown in June.

4. Processes that lead to what appear like symptoms of ageing advance much more rapidly in the June plants. The cotyledons of the June plants change relatively quickly from a green to a yellow-green color and their reserves of nitrogen are withdrawn very rapidly during this time. The cotyledons of the December plants lose their chlorophyll more slowly and there appears to be less modification of the green color previous to its disappearance. Leaves of the May seedlings also soon change from a green to a yellow-green color, the older leaves showing the change first. The green color continues to diminish until none is left. The leaves of the December plants grow for a longer time and the green color persists for a much longer time. During their later development the older leaves become somewhat greyish-green in color, and eventually the green color disappears.

5. The disappearance of green color from the leaves was accompanied by a depletion of the plasma content of the chloroplasts. Leaves of the May and June plants which had lost most of their greenness had many disintegrated chloroplasts, especially in the palisade tissue.

6. Analyses of the nitrogen content of June and December plants yielded evidence which supports the observations made by microscopic examination. Leaves of the December plants contained much more of the total nitrogen of the plants than those grown during May. The May plants contained much more nitrogen in their roots.

7. Leaves of the May plants had shiny upper surfaces, whereas those of the December plants had dull upper surfaces. Leaves of the latter plants also wilted more quickly when removed from the plants.

B. Seedlings Grown With an External Source of Nitrogen

1. The effect of additional nitrogen on the growth of different organs of squash seedlings agrees with results reported earlier for many other types of seedlings, *i.e.*, the growth of tops, particularly of the leaves, is stimulated more than growth of roots.

2. Addition of nitrogen results in increased green and dry weights of all organs but much more in the case of plants grown in June than in December.

3. It appears that shortage of carbohydrates may be a factor which limited the growth of the December plants.

4. The symptoms of early yellowing and dying of leaves of the June plants grown without additional nitrogen were not observed in the plants supplied with nitrogen. Microscopic examination of the leaves corresponding in age to those of the yellowing leaves of the un-nitrated plants showed that the cells of the palisade tissue contained rounded chloroplasts, with a relatively dense plasma content, and bright green in color. These results indicate that a supply of readily available nitrogen is necessary not only for growth of leaves in the light, but also for maintenance, especially if they are exposed to intense illumination for long periods.



REID: NITROGEN METABOLISM

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EXPLANATION OF PLATE XXI

- FIG. 1. Seedling grown in November-December without additional nitrogen.
FIG. 2. Seedling grown in November-December with additional nitrogen.
FIG. 3. Seedling grown in June without additional nitrogen.
FIG. 4. Seedling grown in June with additional nitrogen.

INFLUENCE OF ENVIRONMENT ON THE CALLUSING OF APPLE CUTTINGS AND GRAFTS

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INTRODUCTION

A particular impetus for the study of callus physiology has been given by the results of recent investigations with apple crown gall which tend to show that the larger proportion of overgrowths on root-grafted trees are not bacterial tumors but callus tumors, formed chiefly as proliferations of the scion lip (Melhus, 1926). The fact that overgrowths may be non-parasitic in origin does not minimize their importance, but throws the emphasis of control on the regulation of growth processes of individual plants. This would obviate the consideration of their spread from one plant to another, the problem being rather one of inhibiting the excessive formation of callus cells at the graft union because of the frequent occurrence of overgrowths at this point.

The literature reveals many references to callusing, particularly with respect to the derivation and growth of callus cells and tissues. Of particular importance in this connection are the studies of Trécul (1853), Göppert (1874), Stoll (1874), Küster (1903), and Sorauer (1908). Küster and Sorauer have brought together most of the general information with respect to callus available up to 1900.

Though environmental factors have been studied many times in their relation to plant growth processes in general, the particular influence of these factors on the formation of callus tissue has received scant attention. Küster (1903) pointed out that external conditions influence callusing, and of these moisture is very important. He stated that moisture, supplied either in gaseous or in liquid state, is essential for callus development, and further, that although callus may form under water, its formation is much more abundant in moist air, supposedly because transpiration and the absorption of oxygen are hindered under water. Nutritive conditions were considered by Küster to influence callusing. Organs rich in elaborated food were believed to develop wound tissue more abundantly than those poor in such materials. Under similar external conditions the capacity of both cut ends of a cutting for forming callus was not found to be equal. With poplar cuttings the basal poles callused more abundantly than the apical. Similar results were obtained with *Rosa*. With dandelion roots, callus formed more readily from the upper end, but with root cuttings of *Medicago sativa* more callus developed from the lower or root end. Küster

was unable to decide what factors influenced this polarity, but considered inequalities in nutritive conditions important.

Simon (1908) attempted to control temperature and moisture in his study of callus formation on cuttings of *Populus nigra* and *Populus canadensis*. He found that at 14°–18° C. callus formed after eight days, at 25° after four days, and at 32° after three days. The apical and basal ends of cuttings were found to respond differently to humidity. Whereas apical callusing was greatest at 85 to 90 percent relative humidity, basal callusing was best at humidities between 90 and 94 percent. At complete saturation callusing became more or less equalized after a time for both ends of the cuttings, although at first callus formed more abundantly from the basal end.

Riker and Keitt (1926), in discussing the development of excess callus and wound overgrowth in the case of apple root-grafts, stated that "The influences of temperature and, even more particularly, of moisture are of very great importance in determining the extent of callus development." These authors report studying the influence of temperature and moisture on the development of callus on apple grafts but present no data on this work. They found that "within certain limits callus development increased with higher temperatures and moistures and decreased with lower temperatures and moistures." A relation of food to overgrowth formation is suggested by the statement: "Injuries such as those from cultivators, hoes, hooks, and insects may serve to stop the downward passage of elaborated food and to lead to developments similar to those which follow a poor fit in grafting. Such developments are normal processes of the plants when suitable conditions are provided." And further, in referring to the excessive formation of callus from the scion lip, they say, "It seems probable that the food as it descended the twig to the cut made a slow lateral movement along an obtuse angle to the lower tip where it accumulated and contributed to the development of callus. In a well-fitted graft such an accumulation occurred in a much smaller degree because the food material passed through the united cambium layers into the root." They report that "wound overgrowths have been observed to reach considerable size on some plants and to continue their development for several years."

Rehwald (1927) found that callus formation from root cuttings of *Daucus Carota* was facilitated by water-saturated air. He also found that the top and base of such cuttings differed in their capacity for callus formation, obtaining callus only from the basal portion of the cutting.

Swingle (1929) studied the relation of temperature, water, and oxygen to apple root growth and callus development. He used chiefly stem cuttings of the apple variety Springdale and the willow (*Salix alba* L.). The apple cuttings were all obtained from a single tree, representing wood from three to ten years old. Callusing was usually determined for a ten-day period only. In these experiments temperature appears to have been

well controlled. Moisture was supplied in two ways: either the cuttings were held in a saturated atmosphere, or during the callusing period or previously they were partly or entirely immersed in water. In the study of aëration, oxygen was supplied in three concentrations: 5 percent, 20 percent (air), and pure oxygen, these gas mixtures being passed through the control chambers at different rates of flow. Swingle concluded that a preliminary treatment of the plant material with water retarded callusing. Callusing seemed to be more active with slightly higher temperatures and with somewhat lower water and oxygen supplies than were indicated for the most active production of roots. Under good environmental conditions he found callus formation to be more active at the basal end of the cuttings, suggesting an internal polarity. Oxygen (100 percent) at a pressure of one atmosphere distinctly retarded callus formation.

Hitchcock (1928), in studying rooting response, found that good callus formation occurred on many hard-wood cuttings in a peat moss medium of low moisture content (140 percent).

Kostoff (1928) studied the graft unions of intergeneric and interspecific crosses between members of the Solanaceae. He came to the conclusion that the callus tissues joining scion and stock are chiefly the product of the stock. Various types of tumors superficially like crown gall were observed immediately above the callus. Microscopical examination showed that large quantities of starch, produced by the scions, had accumulated just above the callus due to the fact that the union intercepted their passage. This large accumulation of food was considered the specific cause of the proliferations.

Miss Smith (1928), working with *Clematis*, found that the "amount of callus formed by stem cuttings varies with the age of the wood taken, the amount of food reserves, as well as with the anatomical structure of the species," but that "other factors no doubt come into play." She states further that "There is an undoubted correlation between the amount of starch present in the tissues and the amount of callus formed by any given cutting, though it is not yet possible to express it as a quantitative relation." Different species of *Clematis* were found to vary in degree of callus formation. Under similar temperature conditions (bottom heat of 18.3° C.) *C. afoliata*, *C. smilacifolia*, and *C. uncinata* produced callus slowly and the total amount formed was small, while such species as *C. Armandi*, *C. Hillarii*, and *C. ranunculoides* produced large calluses at a comparatively rapid rate. She pointed out that following the cutting injury to a stem there are death-changes in the divided protoplasts due to the altered metabolism of the injured cells. Following these death-changes a suberin seal is laid down, and still later the first signs of abnormal cell divisions may be seen. These processes were described for *C. smilacifolia*.

The present study was undertaken for the purpose of obtaining quantitative information regarding the influence of such primary environmental

factors as temperature and moisture on the callusing of apple root-grafts. The broad application of such information to horticultural practices is obvious. It was further considered possible that a greater knowledge of environmental influences on callusing might throw light on the problem of how these conditions may be manipulated to favor or restrict excessive callus formation, and that detailed observations on the callusing of scion and stock under many varied conditions might contribute toward a better understanding of the factors influencing the production of overgrowths.

MATERIALS AND METHODS

The study was begun at the Boyce Thompson Institute for Plant Research during the spring of 1927 and was continued for two years. The early part of the work was devoted to a preliminary survey of the comparative importance of many different environmental factors on callusing in addition to a search for satisfactory methods of obtaining quantitative information on the effect of certain conditions. A general discussion of the materials used and the methods followed is presented herewith, and detailed procedures are given later for each individual experiment.

Plant Material.—With only a few exceptions, one-year-old shoots and one-year-old seedling root-stocks were employed. The scion material consisted of standard straight whips grown in a scion orchard, and represented the following varieties: Jonathan, Wealthy, Yellow Transparent, Ben Davis, Grimes, Delicious, Wolf River, Willow Twig, Northwestern, and Winesap. Scions were obtained chiefly from the Mount Arbor Nursery, Shenandoah, Iowa, though some were secured from the Chase Brothers Company, Rochester, New York. Occasionally material of unknown variety was used; this was obtained from trees located on the premises of the Boyce Thompson Institute, Yonkers, New York.

A number of kinds of root-stock material were used, including French crab, Kansas grown; French crab, French grown; Austrian crab, California grown; seedlings of northern hardy varieties, Minnesota grown; and Tennessee crab, Kansas grown. Later the following were added to the list: Vermont crab, Kansas grown; seedlings of northwestern varieties, Washington grown, as well as the Hopa and Meador's winter flowering crabs, Kansas grown. These stocks were obtained from J. H. Skinner and Company, Topeka, Kansas; Vistica Nurseries, Inc., Stockton, California; Oliver Nursery Company, Topeka, Kansas; Clinton Falls Nursery Company, Owatonna, Minnesota; Washington Nursery Company, Toppenish, Washington; and Chase Brothers Company, Rochester, New York.

Preparation of Plant Material.—The scion and root-stock material was callused both as grafts in which the two symbionts were combined (Pl. XXII, figs. 1 and 2) and as cuttings in which they were separate (Pl. XXIII, fig. 6). In either case the individual pieces were cut as they would be in making a tongue graft; that is, a diagonal cut about 3 centimeters ($1\frac{1}{4}$ inches)

in length was made on the bottom end of the scion cutting, and a similar cut was made on the top end of the root cuttings (bottom in this sense meaning downward and top upward with respect to the ground line.) All cuts were carefully made with a sharp grafting knife. Grafts were not used entirely because of the great difficulty of measuring the callus of a graft union. In those experiments in which grafts were used, a sufficient number of individuals was taken so that after breaking the union apart and examining the callus formed, the graft could be discarded. Many tests have indicated that the influence of the environment on callus formation at the union of a graft could be determined just as accurately with the scion and stock separate as with them together. In some of the later experiments both ends of the cuttings were cut off squarely with hand shears, and then the roughly cut surfaces were trimmed with a sharp knife so as to provide a smooth, even surface. It is less difficult to measure the amount of callus in a symmetrical roll than in an asymmetrical roll such as is formed with the slanting cut where each area along the slant may differ in its ability to form callus.

The cuttings usually averaged from 12 to 15 centimeters (5 to 6 inches) in length, scion cuttings usually being longer than stock cuttings. The scion cuttings were cut apically immediately above a bud; at the slanting-cut end a bud was left on the back of the lip, since this is the custom among horticulturalists. Tongue grafts were usually employed for the callusing experiments, although wedge grafts and modifications of both tongue and wedge grafts were used from time to time. The procedure for making a tongue graft is so well known that it need not be repeated here.

The plant material was never allowed to dry. Before being cut up for the experiments, the shoots and root-stocks were stored in moist peat moss at a temperature of about 3° C. In this way the plant material has been kept in satisfactory condition for nearly two years. After cutting, special care was taken to prevent the cut surfaces from becoming injured by drying. In some instances when the cuttings were to be used immediately, they were dropped into a pan of water, but the more common procedure was to place them in moist peat moss until the experiment was begun. Experiments requiring only a small amount of plant material were usually begun the same day that the material was cut. For larger experiments, requiring from several hundred to several thousand cuttings or grafts, the material was placed in moist peat moss at 3° C. as soon as prepared until all pieces could be started simultaneously.

Temperature Experiments.—For the study of the influence of temperature several types of equipment were used, including (1) electrically-heated ovens, (2) refrigeration rooms, (3) temperature-controlled greenhouses, (4) basement storage room, (5) outdoor storage cellar, and (6) cold frame. Each type has its advantages as well as its limitations, but the combined conditions that were available permitted the study of constant and variable temperatures, of slowly and sharply fluctuating temperatures, and allowed

the use of small or large amounts of plant material, together with such apparatus as was required.

The controlled ovens ranged in temperature from 0° to 40° C., with a separate oven for each 5° between these two extremes. The refrigeration rooms were operated at 3° , 10° , 15° , and 20° C. Temperatures in the greenhouses showed considerable fluctuation over the 24-hour period, but the automatic steam control prevented a drop in temperature below any specified point. Both the basement storage room and the outdoor storage cellar maintained a fairly even temperature, but both were subject to the influence of any prolonged change in outdoor temperatures. The temperature of the cold frame varied almost in accordance with that outside.

Moisture Experiments.—To procure and maintain a graded series of moisture conditions was not easily accomplished. Several methods were tried, no one of which was wholly satisfactory. The control of relative humidity was attempted chiefly in two ways: by means of sulfuric acid solutions, and by means of saturated solutions of inorganic salts. Each of these methods presents difficulties. The sulfuric acid solution becomes more and more dilute as it takes up water from the surrounding plant material. As a result the concentration and corresponding vapor pressure change. Additional acid must be added to restore the original concentration. The saturated salt solution has the advantage that it is self-regulatory as to vapor pressure. As water vapor is taken up by the solution the undissolved crystals go into solution, serving to keep the concentration uniform.

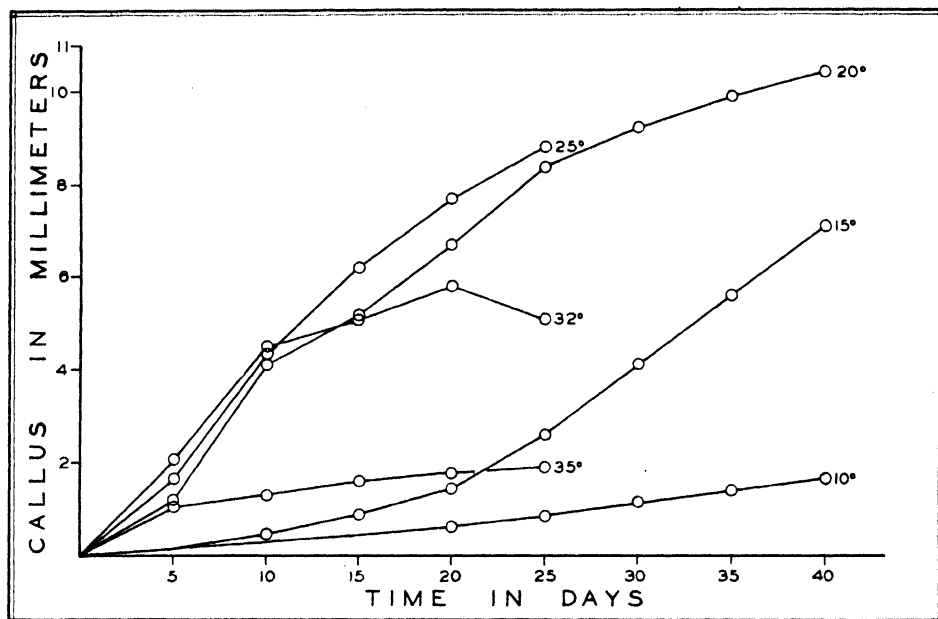
Open systems (in which fresh air was constantly supplied) and closed systems (using stoppered chambers) were used. These systems were operated at various temperatures. With each system sulfuric acid solutions and inorganic salt solutions were used to control vapor pressure. Controlled chambers used in the various tests consisted of test tubes, bottles, desiccators, and crocks up to 12 gallons in capacity. Further, the air in the chambers was circulated by motor-driven fans in some experiments and not in others.

For studies of the influence of relatively high supplies of water, the plant materials were placed in direct contact with peat moss having various water contents. Here little difficulty was encountered. Representative samples of the peat moss were taken from time to time, and the water content was determined by weight. The lack of complete uniformity of moisture in the peat moss and the necessity for taking representative samples comprised the main difficulties; these were, however, not very serious. For these experiments use was made of both covered and uncovered containers, including test tubes of various sizes, desiccators, crocks, and greenhouse flats.

Aëration Experiments.—To study the influence of oxygen on the formation of callus a number of experiments were carried out in which various

mixtures of oxygen with air or nitrogen were used. The oxygen content of the mixtures ranged from 0 to 100 percent. In these experiments a fresh supply of the gas mixture was forced through the control chamber each day. The gases were obtained from cylinders of the Linde Air Products Company, and the required volumes were measured by the displacement of water in a graduated carboy. The prepared atmosphere was then forced through the control chamber by tap-water pressure. By this method the gas mixtures could be prepared with sufficient accuracy for these tests.

Polarity and Variety Experiments.—For experiments on the influence of polarity and variety on callus formation, and in other experiments in which optimum environmental conditions were desirable, moderately moist peat

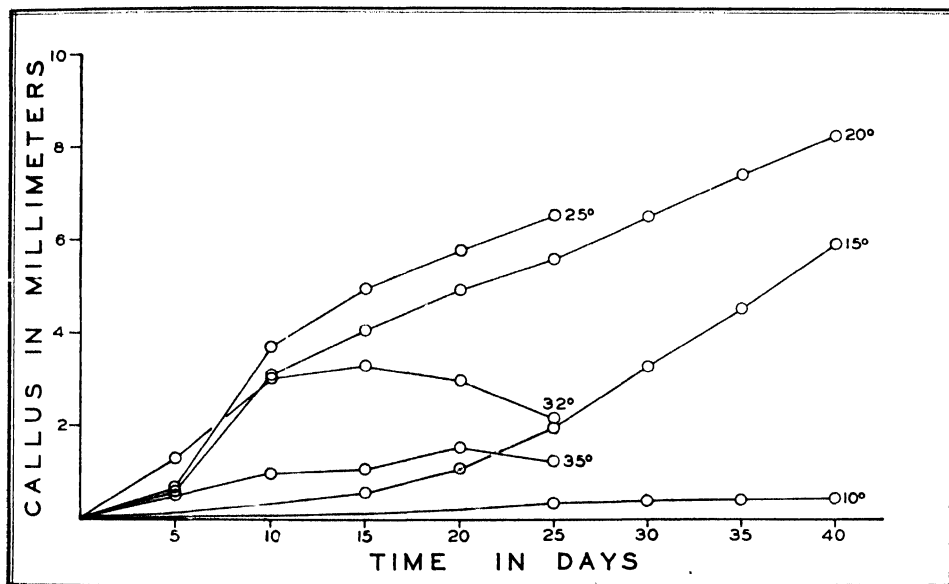


TEXT FIG. 1. Graphs showing the effect of temperature (degrees Centigrade) on the callusing of apple *scion* cuttings. The points of the curves, representing the average diameters of the callus rolls for individual cuttings, are based on four lots of material taken at five-day intervals. No callus formed at 0°, 5°, and 40° C. during the 40-day period.

moss was generally used as a medium. Flats, crocks, or other types of containers were used. The principal requisites for these tests were suitable temperature, moisture, and aëration to permit normal callus formation.

Measuring Callus Development.—In recording the amount of callus formed in the different experiments, readings were generally made on the basis of the diameter of the callus roll in millimeters. Since distinct differences exist in the amount of callus formed at the lip as compared with that formed on the side and base of slanting cuts, separate readings were made for these different areas. It was not considered possible or even essential to make these determinations precise. Two arbitrary values were

used for callus less than one millimeter; namely, a trace, recorded as 0.25 millimeter, and slightly less than one millimeter, recorded as 0.75 millimeter. No fractional values were recorded above one millimeter; whole values were used, as one millimeter, two millimeters, etc. In reading the callusing of a graft union, the graft was pulled apart and the amount of callus on the scion cut and on the stock cut was recorded as for the cuttings. In very advanced stages of callusing, it was sometimes difficult to determine the exact origin (scion or stock) of the callus, for it could not be expected that



TEXT FIG. 2. Graphs showing the effect of temperature (degrees Centigrade) on the callusing of apple root cuttings. The points of the curves, representing the average diameters of the callus rolls for individual cuttings, are based on four lots of material taken at five-day intervals. No callus formed at 0°, 5°, and 40° C. during the 40-day period.

by roughly breaking apart two connecting meristems all the scion callus would adhere to the scion piece and all the stock callus would adhere to the root piece. However, by combining the callus found on the two pieces, the total amount present at the union could be determined with sufficient accuracy.

EXPERIMENTS AND RESULTS

Effect of Temperature on Callus Formation

Experiment 1 (Constant Temperatures)

Methods.—Yellow Transparent scion cuttings and northern hardy seedling root cuttings were callused at the following constant temperatures: 0°, 5°, 10°, 15°, 20°, 25°, 32°, 35°, and 40° C. The purpose of the experiment was to study the specific effect of temperature on callusing. To observe these processes in detail it was necessary to use comparatively small numbers of cuttings; to offset the error that would necessarily result

TABLE 1. *Effect of Constant Temperatures on the Rate of Callusing of Scion and Stock Cuttings at Optimum Moisture (Callus per Individual Cutting as Total of Diameters of Roll Formed on Lip, Side, and Base of Slanting Cut)*

| Time in Days | 0° C. | | 5° C. | | 10° C. | | 15° C. | | 20° C. | | 25° C. | | 32° C. | | 35° C. | | 40° C. | |
|--------------------|-------|-------|-------|-------|--------|-------|----------|-------|----------|-------|---------|-------|---------|-------|--------|-------|--------|-------|
| | Scion | Stock | Scion | Stock | Scion | Stock | Scion | Stock | Scion | Stock | Scion | Stock | Scion | Stock | Scion | Stock | Scion | Stock |
| 3 | | | | | | | | | | | | | | | | | | |
| 4 | | | | | | | | | (c) .25 | .75 | (d) .75 | 0 | (a) 2.5 | .75 | .75 | .75 | | |
| 5 | | | | | | | | | (b) .75 | .75 | 1.25 | .75 | .75 | .75 | .75 | 0 | | |
| | | | | | | | | | (d) .75 | .50 | 2.25 | .75 | 2.25 | 1.75 | 2.25 | .75 | | |
| 6 | | | | | | | | | | | | | | | | | | |
| 7 | | | | | | | (a) .25 | 0 | (c) 2.25 | .75 | .75 | .75 | 2.0 | 1.50 | 0 | 0 | | |
| | | | | | | | | | (b) 3.0 | .75 | 3.5 | .75 | 4.0 | 4.0 | 1.5 | 1.5 | | |
| 8 | | | | | | | | | (d) 2.25 | 2.25 | 2.5 | 2.25 | 4.75 | 1.75 | 1.5 | .75 | | |
| 9 | | | | | | | | | (c) 2.0 | 1.25 | 2.25 | 2.25 | 2.25 | .75 | .75 | .5 | | |
| | | | | | | | | | (c) 3.0 | 4.75 | 2.25 | 4.0 | 4.0 | 2.0 | 0 | 0 | | |
| 10 | | | | | | | (a) .75 | 0 | 3.0 | .75 | 3.75 | .75 | 7.0 | 6.0 | 2.0 | 1.75 | | |
| | | | | | | | (b) .25 | 0 | 6.0 | 3.0 | 4.0 | 5.0 | 4.25 | 2.25 | 2.0 | 1.5 | | |
| | | | | | | | | | (d) 3.5 | 1.75 | 4.75 | 2.25 | 2.75 | .5 | .5 | .5 | | |
| 11 | | | | | | | (a) 1.25 | .5 | 4.0 | 5.0 | 5.0 | 5.0 | 7.0 | 6.0 | 2.0 | 2.0 | | |
| 12 | | | | | | | (c) .25 | 0 | 4.0 | 4.75 | 5.0 | 5.0 | 4.0 | 4.0 | 0 | 0 | | |
| 14 | | | | | | | (b) .25 | 0 | 6.0 | 3.0 | 5.0 | 5.0 | 4.25 | 2.25 | 3.25 | 1.5 | | |
| 15 | | | | | | | (a) 1.5 | .75 | 5.0 | 5.25 | 6.0 | 6.75 | 8.0 | 6.75 | 2.0 | 2.0 | | |
| | | | | | | | (d) 0 | .25 | 5.0 | 1.75 | 7.0 | 2.5 | 2.75 | .5 | 1.5 | .5 | | |
| 16 | | | | | | | (c) .25 | .75 | 4.0 | 4.25 | 5.0 | 4.75 | 4.0 | 4.0 | 0 | 0 | | |
| 17 | | | | | | | (b) 1.5 | 0 | 7.0 | 6.0 | 7.0 | 6.0 | 6.25 | 2.25 | 3.0 | 1.5 | | |
| 19 | | | | | | | 2.0 | 1.5 | 6.0 | 5.75 | 9.0 | 7.0 | 10.0 | 6.0 | 2.25 | 4.0 | | |
| 23 | | | | | | | | | | | | | | | | | | |
| 24 | | | | | | | (c) .75 | 1.75 | 9.0 | 2.5 | 9.0 | 6.0 | 4.0 | .5 | 1.5 | 1.5 | | |
| 25 | | | | | | | (c) .5 | 1.5 | 7.0 | 4.75 | 7.0 | 6.0 | 4.0 | 4.0 | 0 | 0 | | |
| 27 | | | | | | | 3.5 | .5 | 8.0 | 9.0 | 8.0 | 6.0 | 7.0 | 1.5 | 4.0 | 1.5 | | |
| | | | | | | | 5.0 | 4.0 | 10.0 | 5.25 | 11.0 | 7.0 | 4.0 | 1.75 | 2.0 | 1.0 | | |
| 39 | | | | | | | | | | | | | | | | | | |
| 40 | | | | | | | (d) .75 | .5 | 12.0 | 9.0 | | | | | | | | |
| 41 | | | | | | | (c) 2.25 | .25 | 10.0 | 9.0 | | | | | | | | |
| 43 | | | | | | | (b) 2.25 | .25 | 10.0 | 9.0 | | | | | | | | |
| | | | | | | | (a) 2.25 | .75 | 10.0 | 6.25 | | | | | | | | |

(a), Lot of 12/31/27. (b), Lot of 1/1/28. (c), Lot of 1/2/28. (d), Lot of 1/3/28.

from using a single cutting for each temperature, a similar series was started on each of four successive days. The containers were 21-centimeter ($8\frac{1}{4}$ -inch) test tubes, and into each tube filled with moist peat moss one cutting was placed so that it was completely covered with the medium. The tube was then closed with a cork stopper having a very small aperture to permit some exchange of air. One tube for each scion and root cutting was placed at each of the nine temperatures, thus making a series of 18 tubes in all. One series was started on December 31, the second series on January 1, the third on January 2, and the fourth on January 3.

Examinations were made after 4, 7, 9, 11, 14, 19, 27, and 43 days. Readings were made very rapidly so as to reduce to a minimum the injury that might have resulted through exposure to the air. At each examination the general condition of the plant material was noted, as well as the amount of callus formed on the lip, side, and top of the slanting cut.

Results.—Table 1 shows the amount of callus formed per cutting, each value in the table having been obtained by totalling the diameters of the callus rolls on the lip, side, and base of the slanting cut. Averages for the four different lots at five-day intervals are plotted in text figures 1 and 2. It may be seen from table 1 that at 5° C. or below no callus formed during the 43-day period of the experiment. At 10° measurable callus had formed on the scion after 19 days and on the stock after 23 days, the amount increasing during the following three weeks at a fairly slow, uniform rate. At 15° callusing began after seven days on the scion and after 11 days on the stock. As shown in text figures 1 and 2, at 15° there was a gradual growth acceleration during the period, and by the 40th day the growth curve was still upward. At 20° , 25° , and 32° measurable amounts of callus had formed during the first five days. At these three temperatures growth was very rapid during the early part of the growth period; the rate at 25° was somewhat higher than at 20° , and the rate at 32° was slightly higher than at 25° . During the 40-day period the amount of callus reached a maximum and then decreased at both 25° and 32° , whereas the amount of callus continued to increase at 10° , 15° , and 20° . The time before the maximum volume was attained was shorter with a higher temperature. Callusing reached its greatest volume in approximately 20 days at 32° and 25 days at 25° . At 35° callus began to form promptly; it continued to increase slowly in volume for the first three weeks, but soon became browned on the surface and showed signs of injury. No callus was ever observed to form at 40° C.

Discussion.—The effect of temperature on the rate of callus formation is very striking. Temperatures falling between 0° and 40° C. represent the range of possible temperatures at which callus may form on detached shoot and root cuttings of the apple. It is very doubtful if callus ever would form at 0° with this type of material. At 5° no appreciable amount of callus formed during a period of approximately two months, but over a

period of from six months to one year distinct callusing did occur at this temperature (not shown in these data). At 10° callusing began very slowly, and after starting, usually showed little or no acceleration; there was merely a steady increase in volume of callus. For storage periods between one and two months 15° was found to be a good callusing temperature. At this temperature callus formation began more slowly than at higher temperatures but more rapidly than at 10° , and after an initial period of slow growth, a distinct acceleration occurred. (The characteristic appearance of grafts stored for six or seven weeks at 5° , 10° , and 15° C. is shown in Plate XXII, figure 2.) Rapid callusing occurred at 20° , 25° , and 32° , the rate being more rapid the higher the temperature. A maximum volume was attained at 25° after about four weeks, and at 32° after from two to three weeks; the volume then decreased at each of these temperatures. While the data suggest that a greater final volume of callus may be had at 20° or lower than at higher temperatures, this may not always be the case. A preferable interpretation of repeated trials would be that for callusing periods longer than two weeks, temperatures of 20° or lower will in general give callus that is healthier in appearance than that which develops at higher temperatures, and this improved condition of the callus may often result in greater abundance. At 35° callusing was never found to be satisfactory; the surface cells of the callus became brown and formed a layer of cork almost immediately, so that further growth of necessity must have come from within. At 40° death of the tissues occurred, being consistently followed by an abundance of mold.

For temperatures between 5° and 32° C., and for the initial part of the growth period, the rate of callus formation is greatly accelerated by a rise in temperature. In this respect the growth of callus resembles many chemical processes in which a rise in temperature of 10° C. doubles or trebles the rate of reaction. But for other temperatures, especially those above 32° C., and for the later phases of the growth period, no simple relation exists between temperature and rate of callus formation. Only within certain limits of time and temperature is the growth of callus accelerated with temperature rise and retarded with temperature fall.

In controlling the rate of callus formation by means of temperature, the result may more readily be understood if a callus roll be regarded as a colony of meristematic cells which undergoes a growth cycle like that of a colony of bacterial cells or like that of the plant of which the callus is a part. There are perhaps several stages of callus growth, as Buchanan (1918) has shown for a bacterial culture: an "initial stationary phase," a "positive growth acceleration phase," a "logarithmic growth phase," a "phase of negative growth acceleration," a "maximum stationary phase," and several death phases. While different temperatures specifically influence the duration of these different stages of growth as well as the rate of growth during each phase, the important point to be noted here is that

the formation of new callus cells does not continue indefinitely. A certain maximum volume of callus is reached, following which the callus tissues either disintegrate or are transformed into permanent tissues. With high temperatures this phase is attained very quickly, and unless the temperature is immediately lowered, injury and subsequent decay follow. In the field the plant as a whole grows concurrently with wound healing processes, and instead of decaying, the callus becomes protected by an external corky layer, internal growth continues, and the meristematic cells differentiate into permanent tissues. The result is frequently a callus overgrowth.

Experiment 2 (Constant Temperatures)

Methods.—Wealthy cuttings were placed for a nine-day period at constant temperatures similar to those used in the previous experiment. Two sets of nine 500-cc. wide-mouth bottles were used as containers. In one set moistened filter paper was placed on the bottom of the bottles to maintain a high humidity; in the other, the cuttings were completely surrounded with moist peat moss. To permit an exchange of air the bottles were stoppered with absorbent cotton. In the filter-paper series it was possible to measure the amounts of callus formed without disturbing the cuttings; hence an examination of these was made after four days and an examination of both lots after nine days.

Results.—As shown in table 2, no callus formed at 0°, 5°, 10°, and 40° C. during the nine-day period. At 15° and 20° no callus formed during the first four days, but by the ninth day slight callusing had taken place at 15°

TABLE 2. *Effect of Constant Temperatures on the Rate of Callusing of Scion Cuttings at Optimum Moisture (Average Diameter of Callus Roll per Individual Cutting)*

| Temperature ° C. | Average Callus per Cutting in Millimeters | |
|---------------------|---|----------------|
| | After 4 Days * | After 9 Days † |
| 0..... | 0 | 0 |
| 5..... | 0 | 0 |
| 10..... | 0 | 0 |
| 15..... | 0 | 1.25 |
| 20..... | 0 | 3.25 |
| 25..... | 1.00 | 4.50 |
| 32..... | 1.00 | 5.00 |
| 35..... | 2.00 | 1.75 |
| 40..... | 0 | 0 |

* Filter paper series.

† Averages of both series.

and moderate callusing at 20°. At 25°, 32°, and 35°, callusing had occurred by the fourth day, being most rapid in its formation at the highest of these temperatures. By the ninth day, however, callusing at 25° and 32° had greatly increased, whereas at 35° injury had resulted in an actual decrease in callus.

Discussion.—While the results of experiment 2 covering the nine-day

period are not identical with those of experiment 1, they are sufficiently in accord to show the same general effect of temperature on the rate of callus formation. The time required for initiation and first appearance of callus shortens and the rate of subsequent formation increases with increasing temperature within the limits favorable for tissue growth. Above 32° injury occurred even during this short time.

Experiment 3 (Variable Temperatures)

Methods.—Scion cuttings, root cuttings, and grafts of three apple varieties were callused at variable temperatures. The temperatures used were as follows: (a) 6°–14° C. (av. 9°), slowly rising temperature (outdoor storage cellar); (b) 13°–18° C. (av. 16°), slowly rising temperature (basement storage room); (c) 4°–19° C. (av. 7°), sharply fluctuating temperature (cold frame); (d) 14°–23° C. (av. 18°), sharply fluctuating temperature (greenhouse).

The slowly rising temperatures were recorded by a thermograph and showed a gradual rise from the lowest reading to the highest over a period of 52 days. The sharply fluctuating temperatures were recorded by daily readings. In the cold frame at this time of the year (spring) the temperature rose sharply during the short mid-day period and then dropped rapidly again, remaining comparatively low for the balance of the 24-hour period. The average temperature, based on readings taken at approximately nine o'clock each morning, was only 7° C. The greenhouse temperatures were subject to somewhat the same solar influence. Steam heat prevented the temperatures from ever dropping below a fixed point, but about mid-day a rather sharp rise and fall of temperature occurred due to the sun.

Moisture in this experiment may be regarded as fairly constant since all the plant material was placed in peat moss of nearly the same water content. (It is shown in a later section of this paper that the moisture content of peat may vary greatly without an observable effect on the callusing.)

One flat containing the following plant materials in peat moss was subjected to each of the four variable temperatures:

| | | Number of Pieces |
|--------------------------------|---------------------------------|---------------------|
| Jonathan: | Scion cuttings..... | 10 |
| | Grafts (variously wrapped)..... | 30 |
| Wealthy: | Scion cuttings..... | 10 |
| | Grafts (variously wrapped)..... | 30 |
| Yellow Trans- parent: | Scion cuttings | 10 |
| | Grafts (variously wrapped)..... | 30 |
| French crab root cuttings..... | | 10 |

By the term "variously wrapped" is meant that some of the grafts were wrapped with waxed thread, some with clean muslin cloth, and some

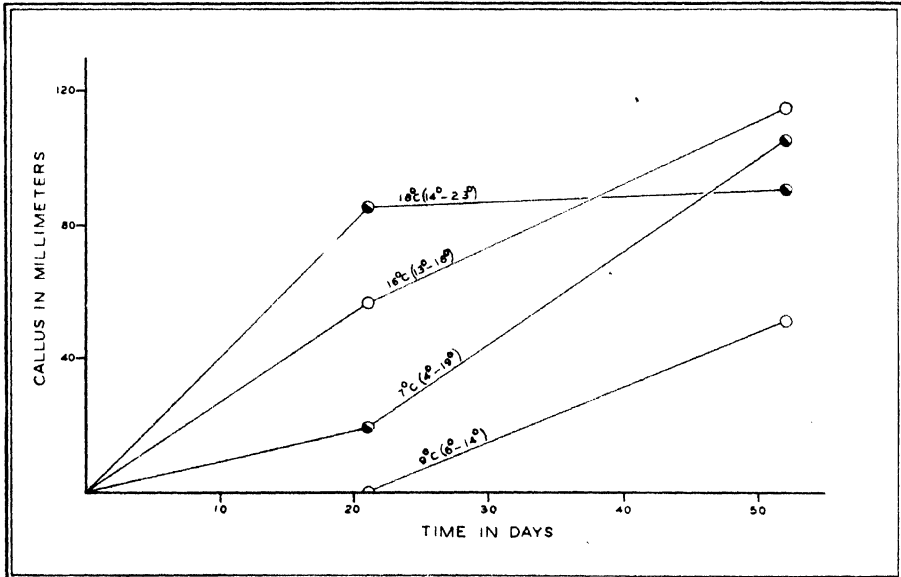
with friction tape. The data for these methods of wrapping are grouped, since the question of wrappers is not to be discussed in this paper.

The material was examined after 21 days and after 52 days. At each of these times from two to five pieces were taken at random from each bundle of plant material. The rest of the bundle was left undisturbed, and the data were recorded rapidly, so as to interrupt growth processes as little as possible.

Results.—Table 3 and text figure 3 show that with the slowly rising temperature beginning at 6°, no callus had formed after three weeks, and that with the corresponding slowly-rising temperature beginning at 13° C. only a moderate amount of callus had formed at this time. However, at

TABLE 3. *Effect of Variable Temperatures on Rate of Callusing of Apple Cuttings and Grafts*

| Time in Days | Total Callus in Millimeters | | | |
|--------------|-------------------------------|------------------|---------------------------------|------------------|
| | Slowly Increasing Temperature | | Sharply Fluctuating Temperature | |
| | 6° C. (6°-14°) | 16° C. (13°-18°) | 7° C. (4°-19°) | 18° C. (14°-23°) |
| 21 | 0 | 57.0 | 19.0 | 86.0 |
| 52 | 54.0 | 115.0 | 104.0 | 91.0 |



TEXT FIG. 3. Graphs showing the effect of variable temperatures (degrees Centigrade) on the callusing of apple cuttings and grafts. The points of the curves are based on the total amount of callus formed, obtained by combining the diameters of the callus rolls for all plant individuals used, the number of pieces for the four different lots being comparable. Both the average temperature for the storage period and the range of variation (in parentheses) are shown.

the end of 52 days, when these temperatures had risen to 14° and 18° C., respectively, moderate callusing had occurred at the lower temperature (about equalling the amount formed at the higher temperature in 21 days), and abundant callusing had occurred at the higher temperature.

With the sharply fluctuating temperature ranging from 4° to 19°, the average temperature was low (7°), but the sudden daily rise in temperature was sufficient to cause a slight callusing after 21 days and abundant callusing after 52 days. In the higher range of sharply fluctuating temperatures (14°–23°) callusing took place under greenhouse conditions, and nearly reached its greatest volume in three weeks.

Discussion.—This experiment again illustrates the point that the rate of callus formation increases with rise in temperature. Within certain limits any rise in temperature appears to accelerate the processes of cell division and cell enlargement and any fall in temperature results in retardation of these processes. Even though a fairly uniform temperature be maintained, any variation from this temperature, even for short periods of time, has a noticeable effect on the volume of callus obtained after a given period of time. The fact that in the range of 14°–23° the amount of callus at the end of 52 days was less than that in the ranges of 13°–18° and 4°–19° was apparently due to unknown conditions in this experiment, and should be regarded as exceptional rather than normal.

Temperature as an Aid in Overgrowth Control

These data not only substantiate the broad generalization that low temperatures retard growth processes and high temperatures accelerate them, but show that callus formation may be regulated as desired by the proper manipulation of temperature. Any tendency toward overgrowth formation during the storage period may be immediately checked by reducing the temperature to about 3° C. This retardation may be only temporary, however, for when the grafts are transferred to field conditions, growth processes may continue. Nevertheless, tongue grafts which have been well callused before planting have only rarely shown the beginnings of an overgrowth at the union after one season in the field. While these grafts were prepared with some care, no attempt was made to select scion and root pieces of equal size or to match precisely the cut surfaces. It may be that a proper regulation of the callusing of root-grafts prior to planting by means of temperature control will prove important in the prevention of overgrowths.

Effect of Temperature and Moisture on Callus Formation

Experiment 4 (Temperature and Moisture)

Methods.—Scion cuttings, root cuttings, and grafts of three apple varieties were callused under conditions of varying temperature and moisture. Averages of the temperatures used were 4°, 9°, and 16° C.

None of these temperatures was strictly constant. The 4° temperature was maintained by refrigeration and occasionally dropped to 0°. The 9° temperature (temperature *a*, experiment 3) was obtained in an outdoor storage cellar, the thermograph record showing a very gradual rise from 6° to 14°. A basement storage room was used for the 16° temperature experiment (temperature *b*, experiment 3), the thermograph record showing a gradual rise from 13° to 18° from March until early June.

Moisture was controlled by means of sulfuric acid solutions, and for each of the three temperatures a series of five humidities was provided, these being expressed as 20, 40, 60, 80, and 100 percent relative humidity. However, in an experiment of this kind the important factor to be controlled is the saturation deficit; that is, the difference between the vapor tension of water at a given temperature and the aqueous tension of a solution of sulfuric acid at that temperature.

Since the vapor pressure curve of water and that of the sulfuric acid solutions diverge from each other as the temperature increases, a solution which will establish a certain saturation deficit at one temperature will no longer do so at another temperature, and in order that the same saturation deficit may be established at both temperatures, solutions of different concentration must be used. The following table shows the saturation deficits corresponding to each of the relative humidities at the three temperatures.

| Percent Relative Humidity | Saturation Deficit (mm. of Hg) ¹ | | |
|---------------------------|---|-------------------|-------------------|
| | 4° C. | 9° C. | 16° C. |
| 20 | 4.88 ^a | 6.89 | 10.90 |
| 40 | 3.66 ^b | 5.17 ^a | 8.18 |
| 60 | 2.44 | 3.44 ^b | 5.45 ^a |
| 80 | 1.22 | 1.72 | 2.73 ^b |

¹ Vapor pressure data from International Critical Tables, volume III. Nearly comparable saturation deficits are provided at different relative humidities, designated by letters *a* and *b*.

Although the experiments were not planned so as to obtain the same series of saturation deficits (from low to high) with each of the three temperatures, nevertheless, for each temperature a considerable range of saturation deficits was provided.

The general procedure was as follows: A large glass crystallizing dish (25 cm. × 12.5 cm.) was placed on the bottom of a 12-gallon glazed crock. Approximately two liters of sulfuric acid solution were placed in the crystallizing dish. The concentrations were carefully prepared with reagent quality acid, according to Wilson's (1921) vapor pressure chart. A circular screen made of heavy galvanized wire was placed on the crystallizing dish, and over this a double thickness of cheese-cloth to prevent soil or plant

particles from falling into the acid. The plant material was then placed in the container. A flanged lid covered the crock and this was sealed airtight with plasteline (modeling wax). Altogether, 15 similar crocks were used to provide the five humidities at three temperatures. An additional crock, in which the plant material was placed over water, was similarly prepared, this crock remaining unopened for 45 days to show if opening the containers to examine plant materials seriously affected the results.

The plant material placed within each crock was as follows:

| | | Number of Pieces |
|--------------------------------|---|---------------------|
| Jonathan: | Scion cuttings..... | 25 |
| | Grafts (variously wrapped) ² | 60 |
| Wealthy: | Scion cuttings..... | 25 |
| | Grafts (variously wrapped)..... | 60 |
| Yellow Trans- parent: | Scion cuttings..... | 25 |
| | Grafts (variously wrapped)..... | 60 |
| French crab root cuttings..... | | 25 |

² See experiment 3.

Examination of the plant material was made at the end of 14, 28, and 49 days. At each of these times the crocks were opened and from two to five pieces taken at random from each bundle. Callusing was examined, observations recorded, and the pieces discarded.

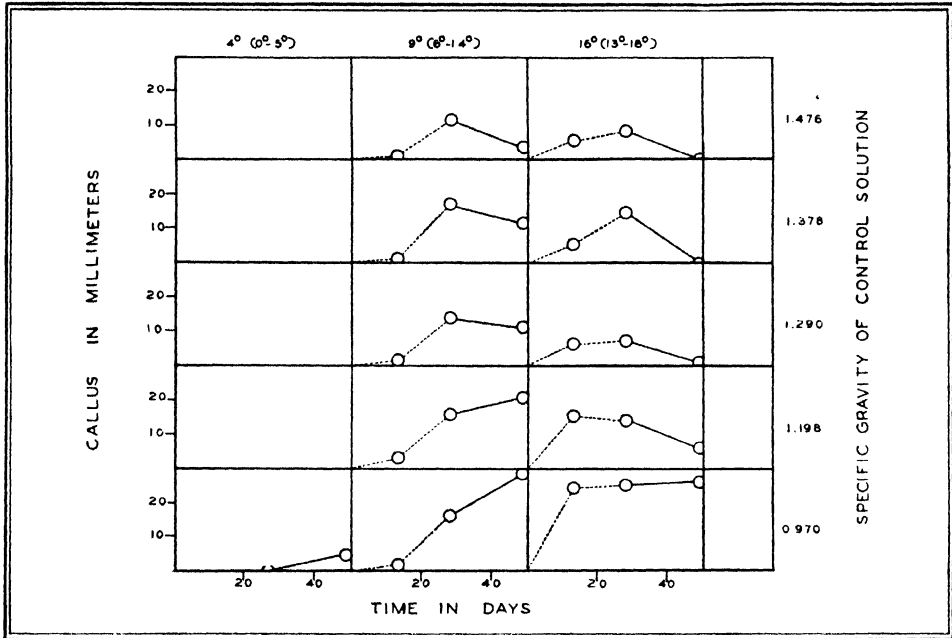
Results.—The amount of callus formed at humidities below saturation was always small, and even at saturation callusing was much less abundant than that obtained in other tests in which the plant materials were in contact with moist peat moss. Table 4 and text figure 4 show that over water at

TABLE 4. *Effect of Temperature and Moisture on the Callusing of Apple Cuttings and Grafts*

| Initial Specific Gravity of Acid | Initial Percent Relative Humidity | Total Callus in Millimeters | | | | | | | | |
|---|--|-----------------------------|------------|------------|----------------|------------|------------|------------------|------------|------------|
| | | 4° C. (0°-4°) | | | 9° C. (6°-14°) | | | 16° C. (13°-18°) | | |
| | | 14 Days | 28 Days | 49 Days | 14 Days | 28 Days | 49 Days | 14 Days | 28 Days | 49 Days |
| 1.476 | 20 | 0 | 0 | 0 | 1.75 | 10.75 | 3.75 | 5.50 | 7.25 | 0 |
| 1.378 | 40 | 0 | 0 | 0 | 0.75 | 16.50 | 11.25 | 5.00 | 14.25 | 0 |
| 1.290 | 60 | 0 | 0 | 0 | 2.00 | 12.75 | 10.50 | 5.25 | 7.00 | 1.50 |
| 1.198 | 80 | 0 | 0 | 0 | 2.75 | 15.25 | 20.25 | 15.25 | 14.75 | 6.75 |
| 0.970 | 100 | 0 | 0 | 4.50 | 2.50 | 17.00 | 27.75 | 24.25 | 25.25 | 25.75 |

4° C. only a trace of callus had been formed at the end of 49 days, and that no callus whatever had been formed at the other humidities. At 9° callusing began very slowly, but after two weeks a sharp acceleration took place due to the steadily rising temperature. In this temperature range slight callusing was had over all acid solutions; but over the three strongest

solutions the callus which had formed during the early period of the experiment was later partially destroyed through desiccation, the rate and extent of desiccation increasing with the acid concentration. Callus formation at the end of 14 days at 16° C. was more abundant in every case than at the



TEXT FIG. 4. Graphs showing the desiccation effect of combined temperatures and humidities on callusing cuttings and grafts. Though slight callusing (totals shown) occurred over acid solutions, particular attention is called to the curves showing decrease in callus due to desiccation (represented by a solid line). The average temperatures (degrees Centigrade) for the storage period and the ranges of variation (in parentheses) are shown. Since relative humidities were not constant in all cases, they are represented by the initial specific gravities of the solutions.

lower temperature ranges, this difference being most pronounced over water and the dilute acid. As in the test at 9°, early-formed callus seems to have disintegrated more quickly with increased concentration of acid. There was little evidence of injury to the callus through opening the containers for examination.

Discussion.—In general, callus formation under the inclosed conditions of this experiment was at the most no more than fair, being neither uniform nor especially abundant in any particular case.

The fact that some callusing did take place over acid solutions of sufficient initial strength to provide very low humidities should not be construed to mean that callus really is able to form if the plant materials are surrounded by air in which these humidities are maintained. It would seem that the explanation lies in failure of the acid to establish an equilibrium quickly. This seems probable from a consideration of the experimental

conditions under which these tests were made, and it is supported by the results of experiment 5, described later. The moisture present in the air of the container at the start was so small in amount that it may be disregarded; a sufficient quantity of it could have been taken up by the acid solution quickly enough to establish an early equilibrium. The plant material placed within the containers, however, was of sufficient quantity to provide a large supply of available water. It seems probable that within a short time considerable water had been evaporated from the plant material and absorbed by the acid solution, and that this water served to dilute the acid solution materially and tended especially to form surface layers of much lower concentration than the underlying mass of solution. In one experiment in which the cuttings were placed over different concentrations of sulfuric acid it was found that at the end of six days they had lost weight as follows:

| Initial Specific Gravity of Acid | Percentage Loss in Weight |
|----------------------------------|---------------------------|
| 1.476..... | 17.2 |
| 1.378..... | 12.2 |
| 1.290..... | 9.6 |
| 1.198..... | 5.7 |
| 0.970..... | 0.9 |

At this rate, estimating the weight of plant material in each container as 2400 grams, 412 cc. of water conceivably may have been withdrawn from the plant material over the most concentrated acid during the first six days. This amount of water would be sufficient to form a watery layer which could greatly modify the drying power of the acid solution. The formation of such a layer would be possible because neither the air in the containers nor the acid solutions was in motion, and considerable time would be required to establish equilibrium through diffusion and convection currents. During this initial period it would be possible that those cuttings most protected by a surrounding mass of plant material could actually callus slightly. If a smaller volume of plant material had been used, and if this material had been separated instead of being placed in bundles in which centrally-located cuttings were more or less protected, it is quite unlikely that callus would have formed over the acid solutions with a specific gravity of 1.476, 1.378, and 1.290.

The effect of these humidities at the different temperatures can be seen more clearly (text fig. 4) where the callus curves show the rate and extent of disintegration rather than the rate of formation and final volume attained. It is likely that equilibrium had been established by that time. During this late phase of the storage period it may be seen that all the slopes of the curves are downward, indicating desiccation, with the exception of the lots in saturated atmospheres and the lot over the most dilute acid at 9° C. All solutions of acid, except in one case, brought about desiccation of the callus of cuttings and grafts, and the rate of desiccation increased

with the acid concentration. Further, desiccation was more marked throughout at the higher temperature.

Experiment 5 (Relative Humidity)

Methods.—Since with sulfuric acid solutions of the preceding experiment the concentrations became modified by the absorption of moisture from the plant material, it seemed desirable to employ other means of maintaining humidity in which this source of error would be eliminated. Hence, an experiment was begun in which apple cuttings were callused in a continuous current of air, the humidity of which was controlled by means of saturated solutions of inorganic salts.

A number of salts were selected to provide a wide range of humidities. All solutions were made of "C.P." salts and distilled water. In the process of preparation, each solution was agitated by a motor-driven stirrer for a period ranging up to four hours in length, while the salt was added as rapidly as it was dissolved. At the same time the temperature of the solution was maintained slightly above that of the room so that on cooling saturation would be assured (one of the salts, calcium sulfate, is slightly more soluble in the cold). The solutions used with the relative humidities delivered, as determined by means of a special humidity testing instrument (Shippy, 1929), were as follows:

| Solution | Percentage Relative Humidity |
|--|------------------------------------|
| $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ | 56 |
| NaNO_2 | 66 |
| NH_4Cl | 79 |
| $(\text{NH}_4)_2\text{SO}_4$ | 81 |
| $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ | 90 |
| $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ | 95 |
| $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ | 96 |
| $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ | 98 |
| Water..... | 100 |

The influence of temperature on vapor pressure and saturation deficit may be disregarded in this experiment, since all relative humidities were provided at the same temperature.

Text figure 5 shows the arrangement of the apparatus. Ten series of four bottles each (one series for each solution) were so arranged that they could be connected to a single vacuum outlet. Three of the bottles were of 500 cc. capacity and each contained about 375 cc. of the solution. The fourth bottle held the cuttings and was of one liter capacity. The vacuum pull was regulated by means of clamps so that a slow, fairly constant flow of air passed through the solutions and containers holding the cuttings.

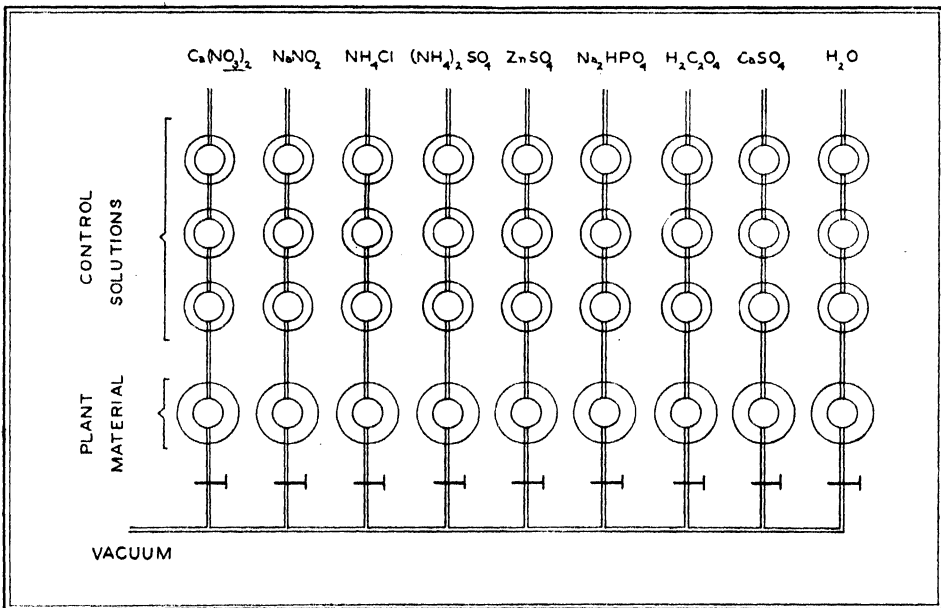
Plant material for the experiment consisted of ten Wealthy apple cuttings for each humidity, making a total of 90 cuttings. Callusing was recorded after six days at room temperature (20° – 25° C.).

Results.—As shown in table 5, during the six-day period the most callus

TABLE 5. *Effect of Relative Humidity on Callusing of Wealthy Cuttings*
(Temperature 22°–25° C.)

| Solution | Percent Relative Humidity | Comparative Amount of Callus |
|-------------------------|---------------------------------|------------------------------------|
| Calcium nitrate..... | 56 | 0 |
| Sodium nitrite..... | 66 | + |
| Ammonium chlorid..... | 79 | + |
| Ammonium sulfate..... | 81 | ++ |
| Zinc sulfate..... | 90 | ++ |
| Disodium phosphate..... | 95 | +++ |
| Oxalic acid..... | 96 | +++ |
| Calcium sulfate..... | 98 | +++ |
| Water..... | 100 | +++ |

+ Trace.
++ More.
+++ Most.



TEXT FIG. 5. Diagram showing the arrangement of equipment for providing a graded series of relative humidities by the use of saturated solutions of inorganic salts. Each vessel (bottle) is represented by two concentric circles, and the tubing used to connect the different series to the vacuum is indicated by solid lines. The small T's show where clamps were attached.

formed between 95 and 100 percent relative humidity, less between 80 and 90 percent, still less between 66 and 79 percent, and no callus formed at 56 percent.

Discussion.—In this experiment, as in the preceding, callus formation was only fair even at humidities close to saturation. Apparently even in

the case of water-saturated air, the moisture condition was not optimum, since at the close of the experiment there appeared no likelihood that the cuttings would callus further.

Experiment 6 (Moisture Toleration)

Methods.—Since moistures below 100 percent relative humidity had failed to give uniform, abundant callusing, in this experiment 100 percent relative humidity was provided in every case and the moisture variation was that of the total water content of the callusing medium (peat moss). Hence, this experiment represented a test under variable temperatures of the tolerance of callus to environments in which liquid water was increasingly available.

Temperatures of 10°, 15°, 20°, and 22°–25° C. were used. The water content of the peat moss medium in which the cuttings were placed varied as follows: 97, 177, 271, 337, and 437 percent (averages for period on basis of 100 parts oven-dried peat moss). The plant material was as follows:

Scion cuttings:

Yellow Transparent
Willow Twig
Wolf River
Ben Davis
Delicious

Root cuttings:

Austrian crab

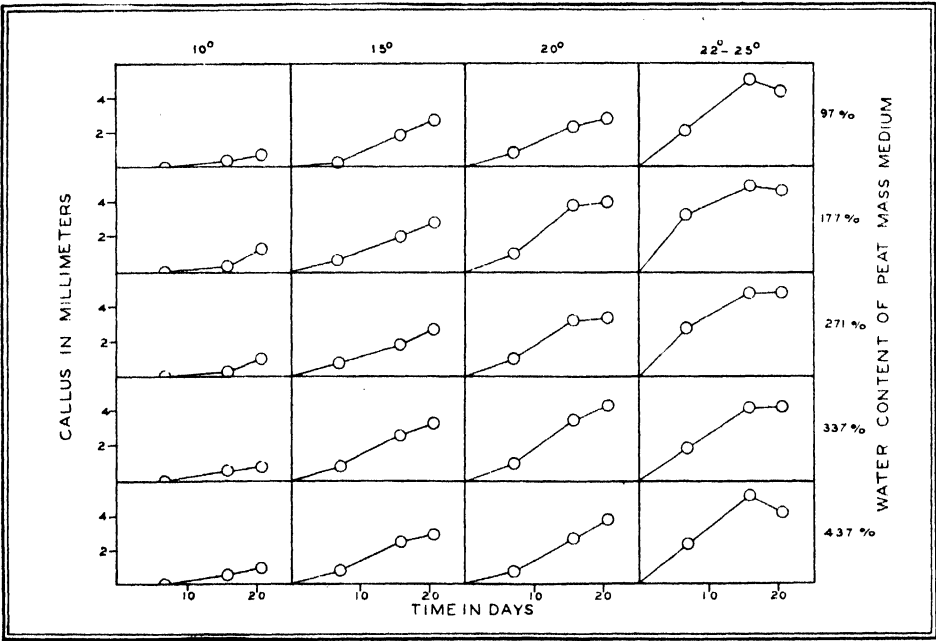
The four temperatures and the five moistures made a total of 20 different sets of conditions. Fifteen cuttings of every variety were used for each condition. All cuttings were first cut with hand shears, and then the ends were trimmed with a sharp knife to ensure a smooth surface. Containers consisted of deep flats (depth, 4 inches) divided into three compartments by lengths of lath. In preparing a flat, a one-inch layer of peat moss was spread over the bottom; the cuttings were placed upon this, and then were covered with enough peat moss to fill the flat. Fifteen flats were used for each temperature, or three for each of the five different moistures.

Examinations were made after 7, 16, and 21 days. Usually three cuttings were picked at random for callus readings, the others remaining undisturbed.

Results.—Table 6 and text figure 6 show that the moisture content of the peat moss medium may vary between wide limits without influencing the rate of callus formation. Considerable moisture tolerance is indicated by the fact that callus formed almost equally well in media varying in water content from 97 to 437 percent (Pl. XXIII, figs. 1–5). Further, these results indicate that once the moisture content of the medium is such that the air within the medium is saturated, additional quantities of water have little or no stimulating effect on callus formation.

TABLE 6. *Effect of Temperature and Moisture on Callusing of Apple Cuttings*

| Water Content of Peat Moss Medium, Percent | Average Callus per Cutting in Millimeters | | | | | | | | | | | |
|--|---|------------|------------|-----------|------------|------------|-----------|------------|------------|------------|------------|------------|
| | 10° C. | | | 15° C. | | | 20° C. | | | 22°-25° C. | | |
| | 7 Days | 16 Days | 21 Days | 7 Days | 16 Days | 21 Days | 7 Days | 16 Days | 21 Days | 7 Days | 16 Days | 21 Days |
| 97 | 0 | .4 | .70 | .25 | 1.9 | 2.8 | .8 | 2.3 | 2.9 | 2.2 | 4.8 | 4.4 |
| 177 | 0 | .4 | 1.25 | .70 | 2.0 | 2.9 | 1.0 | 3.8 | 4.0 | 3.2 | 4.8 | 4.6 |
| 271 | 0 | .4 | 1.70 | 1.0 | 1.7 | 3.0 | 1.0 | 3.2 | 4.3 | 2.7 | 4.7 | 4.7 |
| 337 | 0 | .5 | .90 | .80 | 2.5 | 3.3 | 1.0 | 3.4 | 4.4 | 1.8 | 4.2 | 4.3 |
| 437 | 0 | .5 | 1.00 | .80 | 2.6 | 3.0 | 1.0 | 2.6 | 3.8 | 2.4 | 5.2 | 4.4 |



TEXT FIG. 6. Graphs showing the combined effect of temperature and liquid moisture on the callusing of apple cuttings. The amount of callus is expressed as the average diameter of the callus rolls per individual cutting. Temperature is shown in degrees Centigrade, and moisture is expressed as the total water content of the peat moss medium (percentage water by weight per 100 parts oven-dried peat moss).

Discussion.—The conditions afforded in this experiment, as well as others in which cuttings and grafts have been callused in moist peat moss, permitted uniform callusing that had never been obtained when the material was merely exposed to a moist atmosphere even though the air were almost saturated with water vapor. This has seemed to indicate the desirability of actually having liquid moisture in contact with the cuttings, as is the case in a moss or sand medium. The liquid moisture perhaps need be no more than a film, for good callusing has been had repeatedly in a medium

only slightly moist to the touch; in fact, in a medium in which the cuttings have actually decreased in weight due to water loss. However, if the medium is so dry that the loss of water from the cuttings is great, no callus forms. An experiment carried out with cuttings of Jonathan, Wealthy, and Yellow Transparent gives some idea of the water gain or loss from cuttings placed in a peat moss medium (table 7). The moisture contents

TABLE 7. *Water Gain or Loss of Cuttings and Peat Moss Callusing Medium*

| | Initial Percent Water of Peat | Percent Gain or Loss of Water After 33 Days | |
|------------------------|-------------------------------|---|----------|
| | | Medium | Cuttings |
| A. No callus..... | 16 | +22 | -26 |
| | 16 | +19 | -23 |
| | 17 | +21 | -31 |
| | 17 | +16 | -24 |
| | 17 | +13 | -25 |
| | 19 | +13 | -21 |
| | 19 | +15 | -23 |
| B. Good callusing..... | 98 | - 3 | - 3 |
| | 109 | -16 | - 4 |
| | 113 | -14 | - 6 |
| C. Good callusing..... | 230 | -16 | + 3 |
| | 238 | -31 | + 4 |
| | 252 | -43 | + 3 |
| D. Good callusing..... | 495 | -32 | + 5 |
| | 408 | -46 | + 3 |
| | 418 | -52 | + 3 |

- Loss in weight.
+ Gain in weight.

of groups B, C, and D are all sufficiently high to permit callusing, but the low moisture contents of group A so desiccated the cuttings that callusing could not take place. It seems that for normal callusing the cutting material must be held reasonably close to its original moisture content.

Effect of Aëration on Callus Formation

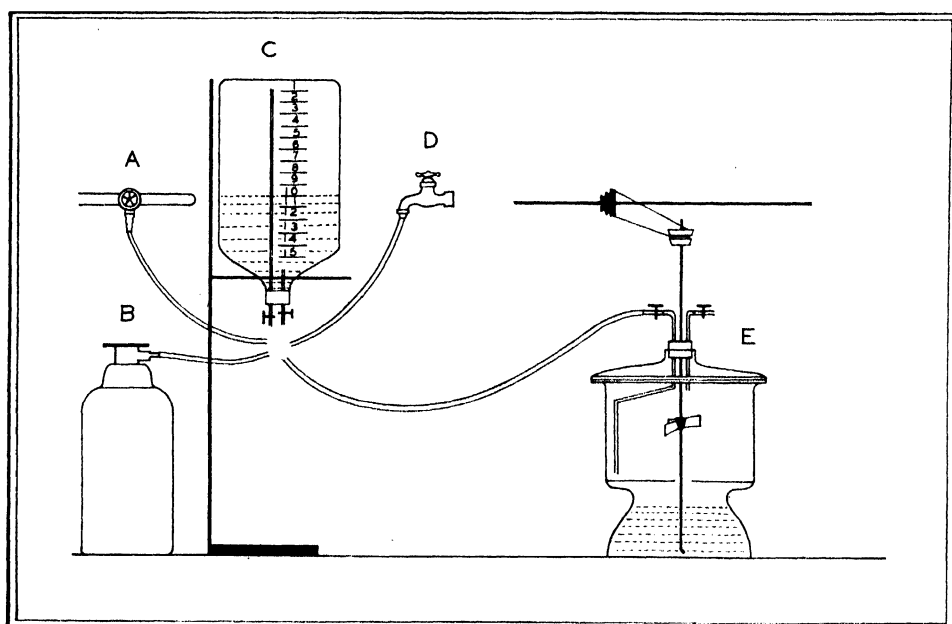
Experiment 7 (Aëration)

Methods.—Cuttings were callused under conditions of varying proportions of oxygen and air, as follows:

Approximate Proportions in Percentage

| Oxygen | Air | | Oxygen | Nitrogen |
|--------|-----|---|--------|----------|
| 0 | 100 | = | 20 | 80 |
| 33 | 66 | = | 46 | 54 |
| 66 | 33 | = | 73 | 27 |
| 100 | 0 | = | 100 | 0 |

Text figure 7 illustrates the apparatus. The cuttings were placed in four large desiccators provided with tubulated covers, closed with three-hole rubber stoppers. Through the central hole a solid glass rod was passed having a fan attachment, which when rotated by means of power supplied by an electric motor, constantly kept the air in motion in the desiccator. Through the other two holes 6 mm. ($\frac{1}{4}$ inch) glass tubing was passed by means of which the atmosphere in the desiccator was changed



TEXT FIG. 7. Diagram showing the arrangement of an apparatus for measuring and transferring to a desiccator various mixtures of oxygen and nitrogen or oxygen and air. The equipment includes (A) compressed air tap, (B) oxygen or nitrogen cylinder, (C) inverted, graduated carboy, (D) water faucet, and (E) desiccator used as control chamber. The proper volume of each gas is admitted to the water-filled carboy through the long tube of the carboy, the displaced water escaping through the short tube. By connecting the short tube with the water faucet and the long tube with that connected with the desiccator, the gas may be forced into the desiccator by tap-water pressure. As shown, the gas enters the lower part of the desiccator, displacing that previously present, which escapes through the short tube at the top.

without disturbing the other contents. Proportions of oxygen and air were measured in a calibrated, inverted carboy by displacing tap water by oxygen from a cylinder of the gas or by air from a compressed air outlet. The gas mixture was then forced into the desiccators by water pressure from the tap. To maintain the original gas mixture ten liters were forced through the desiccators daily in the manner described.

Plant material consisted of apple cuttings of unknown variety cut from trees growing on the premises of the Boyce Thompson Institute. Thirty-four cuttings were placed in each desiccator. Examination was

made after the preparations had remained for five and eight days at room temperature (approximately 25° C.).

Results.—Table 8 shows that callus formed on cuttings placed in high concentrations of oxygen, but indicates that pure oxygen inhibited its formation.

TABLE 8. *Effect of Oxygen on the Callusing of Scion Cuttings*

| Time in Days | Percentage Oxygen | Callus |
|--------------|-------------------|--------|
| 8..... | 20 | ++++ |
| 5..... | 46 | +++ |
| 5..... | 73 | ++ |
| 5..... | 100 | + |

(+) One cutting showing callus.

(++) Several with callus.

(+++) Many with callus.

(++++) Majority with callus.

Discussion.—Callusing was clearly inhibited by 100 percent oxygen. With the other concentrations of oxygen the callusing showed great variability. Although table 8 suggests an inverse relation between callusing and oxygen concentration between 20 and 73 percent, this relation was not confirmed when the tests were repeated, in experiment 8.

Experiment 8 (Aëration)

Methods.—Both apple and privet cuttings were callused under conditions similar in all respects to those of experiment 7. The experimental period was 15 days, and 14 cuttings were used for each gas mixture.

Results.—Table 9 shows that callus may form over a wide range of oxygen concentrations, but pure oxygen again appeared to be inhibiting in its effect. (See Plate XXIII, figure 6, for injury to cuttings exposed to high oxygen concentrations.)

TABLE 9. *Effect of Oxygen on the Callusing of Apple and Privet Cuttings*

| Percentage Oxygen | Callus in Millimeters * | |
|-------------------|-------------------------|--------|
| | Apple | Privet |
| 20..... | 3.3 | 3.0 |
| 46..... | 2.1 | 2.3 |
| 73..... | 3.8 | 2.3 |
| 100..... | 0.8 | 0.0 |

* Average per individual cutting.

Discussion.—In this experiment little difference in volume of callus occurred in oxygen concentrations ranging from that of air to that of an atmosphere containing 73 percent. At 100 percent oxygen, as in the previous experiment, callusing was inhibited.

Experiment 9 (Aëration)

Methods.—Apple cuttings of unknown variety were callused in concentrations of oxygen lower than normal air, as follows:

Approximate Proportions in Percentage

| Air | Nitrogen | | Oxygen | Nitrogen |
|-----|----------|---|--------|----------|
| 0 | 100 | = | 0 | 100 |
| 20 | 80 | = | 4 | 96 |
| 30 | 70 | = | 6 | 94 |
| 40 | 60 | = | 8 | 92 |
| 50 | 50 | = | 10 | 90 |
| 60 | 40 | = | 12 | 88 |
| 70 | 30 | = | 14 | 86 |
| 80 | 20 | = | 16 | 84 |
| 90 | 10 | = | 18 | 82 |
| 100 | 0 | = | 20 | 80 |

The cuttings were placed in calibrated 40 cm. tubes fitted with fine-mesh copper screen supports inside to hold the cuttings above the water level. Cuttings were placed in the tube, and then the copper screen support was forced up into the tube about 5 cm. The tube was filled with water and inverted with the open end held beneath the water. The water was displaced by the proper amounts of compressed air and nitrogen, the tube being so calibrated that a column of water from 2.5 to 5 cm. deep remained inside to supply a high humidity. The tube was closed with a rubber stopper, and then placed at 15° C. The final volume of callus was obtained after 16 days.

Results.—The results given in table 10 show that normal callusing occurs when the oxygen supply is 14 percent or higher. Concentrations of oxygen from 6 percent to 12 percent permitted callus formation and a slight amount of callus formed even in 4 percent oxygen. Any concentration of oxygen below 12 percent was inhibiting in its effects.

TABLE 10. *Effect of Decreased Amounts of Oxygen on the Callusing of Apple Cuttings*

| Percentage Oxygen | Callus in Millimeters * |
|-------------------|-------------------------|
| 0..... | 0.00 |
| 4..... | 0.25 |
| 6..... | 3.00 |
| 8..... | 3.00 |
| 10..... | 3.00 |
| 12..... | 3.00 |
| 14..... | 5.00 |
| 16..... | 5.00 |
| 18..... | 5.00 |
| 20..... | 5.00 |

* Average per individual cutting.

Discussion.—This experiment is subjected to the criticism that since the gas mixture was not renewed from time to time its composition was different at the close of the experimental period from what it was at the beginning.

While this is doubtless true, it is equally safe to say that the oxygen concentrations were never greater than at the beginning of the experiment. These data are in accord with similar experiments showing that callusing does not take place in the absence of oxygen, but that smaller amounts than present in air are sufficient. In the absence of oxygen it is probable that respiration and other metabolic processes are so retarded that cell division, essential for the formation of callus, soon comes to a standstill.

Carbon dioxid, chiefly formed as a product of respiration, must be taken into consideration. In one experiment, cuttings of different ages were placed in pure carbon dioxid as well as in a mixture of equal parts of carbon dioxid and air. In no case (after 18 days) did callus form, whereas moderate callusing took place with similar lots of cuttings in various proportions of oxygen and nitrogen. In another experiment cuttings were placed in a graded series of high carbon dioxid concentrations. After 15 days callus had formed in air and in a mixture containing 10 percent CO_2 and 90 percent air, but no callus had developed in mixtures containing higher percentages of carbon dioxid. Of course, in mixtures of carbon dioxid and air it is possible that oxygen would be a limiting factor. However, it seems more probable that carbon dioxid, in high concentrations, would reduce respiration and thus inhibit callusing.

Effect of Polarity on Callus and Overgrowth Formation

It was early recognized in these experiments that both poles of apple cuttings do not callus equally. It was very evident that the bottom or lower ends of both scion and root cuttings callus the better, and this fact would seem to have an important bearing on the usual formation of overgrowths from the bottom of the scion.

Experiment 10 (Polarity)

Methods.—See experiment 6. It may be noted, however, that the only requirement to illustrate polarity is an environment permitting normal callus formation.

Results.—Distinct polarity was observed in apple scion (Pl. XXIII, figs. 1-5) and root stock cuttings with respect to the amount of callus which formed at opposite ends of the same cutting. Tables 11 and 12 show that callus was formed in much greater abundance on the bottom ends than on the top ends of both scion and root cuttings. This difference occurs regardless of temperature. Not every cutting calluses more abundantly from the bottom end, for individual variations do occur (where some factor, such as injury or localized depletion of water, acts to change the usual result). Observations of hundreds of cuttings, however, leave no doubt as to a marked difference in callusing capacity between the two poles of the same cutting.

TABLE 11. *Abundance of Callus Formed on Top and Bottom Ends of Apple Scion Cuttings **

| Time in Days | Number of Readings | Storage Temperature | Total Callus in Millimeters | |
|--------------|--------------------|---------------------|-----------------------------|--------|
| | | | Top | Bottom |
| 7 | 47 | 15° | 0.25 | 33.25 |
| | 71 | 20 | 3.50 | 58.50 |
| | 63 | 22-25 | 25.75 | 126.50 |
| 16 | 35 | 10° | 0.00 | 18.50 |
| | 72 | 15 | 13.75 | 147.25 |
| | 65 | 20 | 33.75 | 162.50 |
| | 61 | 22-25 | 76.00 | 212.00 |
| 21 | 64 | 10° | 2.50 | 61.50 |
| | 70 | 15 | 30.75 | 172.00 |
| | 71 | 20 | 48.75 | 177.75 |
| | 70 | 22-25 | 85.25 | 182.75 |

* The terms "top" and "bottom" apply with respect to the ground line.

TABLE 12. *Abundance of Callus Formed on Top and Bottom Ends of Apple Root Cuttings **

| Time in Days | Number of Readings | Storage Temperature | Total Callus in Millimeters | |
|--------------|--------------------|---------------------|-----------------------------|--------|
| | | | Top | Bottom |
| 7 | 2 | 20° | 0.00 | 0.50 |
| | 10 | 22-25 | 1.75 | 7.25 |
| 16 | 8 | 15° | 0.50 | 4.25 |
| | 10 | 20 | 2.00 | 9.75 |
| | 10 | 22-25 | 9.75 | 21.50 |
| 21 | 6 | 10° | 0.00 | 3.25 |
| | 13 | 15 | 3.75 | 18.75 |
| | 15 | 20 | 4.50 | 23.50 |
| | 10 | 22-25 | 8.25 | 26.25 |

* The terms "top" and "bottom" apply with respect to the ground line.

Experiment II (Polarity)

Polarity is distinct in the case of the slanting cut made on the basal end of scion cuttings prepared as in making the tongue graft.

Methods.—See experiment. 3. A favorable callusing environment is the only requirement.

Results.—Tables 13 and 14, representing two different tests, show that the lip produces callus far more abundantly than the other areas of the

TABLE 13. *Callus Formation from the Lip, Side, and Top of Slanting Cut Made on Basal End of Scion Cuttings*

| Time in Days | Number of Readings | Total Callus in Millimeters | | |
|--------------|--------------------|-----------------------------|------|-----|
| | | Lip | Side | Top |
| 21 | 48 | 69 | 39 | 41 |
| 52 | 57 | 137 | 63 | 76 |

TABLE 14. *Callus Formation from the Lip, Side, and Top of Slanting Cut Made on Basal End of Scion Cuttings*

| Time in Days | Number of Readings | Total Callus in Millimeters | | |
|--------------|--------------------|-----------------------------|------|-----|
| | | Lip | Side | Top |
| 18 | 95 | 124 | 66 | 64 |
| 52 | 94 | 266 | 112 | 130 |

slanting cut, whereas no marked differences exist between the base and the side. That the dominance of lip callusing is a polarity phenomenon is shown by the fact that similar slanting cuts made on the top end of the root piece show no striking dominance of the lip. In this case the lip, side, and base develop practically the same amount of callus.

Experiment 12 (Polarity)

The question may be raised as to whether or not the position of the cuttings with reference to gravity during the callusing period influences the effects of polarity.

Methods.—Wealthy and Yellow Transparent grafts were callused in upright, horizontal, and inverted positions in a medium of moderately moist peat moss at approximately 20° C. Callusing was examined after 34 days.

Results.—Table 15 shows that callusing was the same, whether the

TABLE 15. *Effect of Position of Apple Grafts During Storage on the Abundance of Callus **

| Variety | Position of Grafts | Scion Callus | | | Root Callus | | |
|--------------------|--------------------|--------------|------|-----|-------------|------|------|
| | | Lip | Side | Top | Lip | Side | Top |
| Wealthy | Upright | 4.0 | 2.0 | 2.0 | 1.0 | 0.75 | 1.0 |
| | Horizontal | 4.0 | 2.0 | 2.0 | 0.75 | 0.00 | 0.75 |
| | Inverted | 4.0 | 2.0 | 2.0 | 1.0 | 0.75 | 1.0 |
| Yellow Transparent | Upright | 4.0 | 0.75 | 2.0 | 0.75 | 0.0 | 1.0 |
| | Horizontal | 3.0 | 2.0 | 2.0 | 1.0 | 0.0 | 1.0 |
| | Inverted | 4.0 | 0.75 | 2.0 | 1.0 | 0.0 | 1.0 |

* Average per individual cutting.

grafts were upright, horizontal, or inverted. The grafts united well in most cases, without reference to position. If gravity were responsible for the basally dominant development of callus, it must have acted prior to the callusing period.

Experiment 13 (Polarity)

Next to the possibility of a gravitational causation for basal dominance, that of food accumulation might be considered. This has not been given particular study in these experiments, although the results of a simple test made in this connection may be of interest.

Methods.—Apple cuttings of current season's growth and of one-year-old and two-year-old wood were used. The basal end of each cutting was treated with a solution of iodine and potassium iodid in water. The cuttings were then divided into two groups, according to whether they contained little or much starch. The treated surfaces were carefully cut away with a sharp knife, and each lot of cuttings was placed in a desiccator over water. The material was sprayed every day with water from an atomizer so as to assure further favorable moisture conditions. Readings were made after 20 days.

Results.—Table 16 shows that the difference in starch content of the cuttings was not correlated with any apparent difference in the abundance of callusing.

TABLE 16. *Influence of Starch Content on Amount of Callus*

| Age of Wood | Number of Pieces | Average Callus per Individual Cutting | | | | | |
|----------------------------|------------------|---------------------------------------|------|------|---------------------------------|------|-----|
| | | Cuttings of Low Starch Content | | | Cuttings of High Starch Content | | |
| | | Lip | Side | Top | Lip | Side | Top |
| Current season's | 7 | 5.0 | 1.0 | 0.75 | 3.0 | 1.0 | 1.0 |
| 1-year-old | 11 | 3.0 | 1.5 | 2.0 | 3.0 | 1.0 | 1.0 |
| 2-year-old | 5 | 3.0 | 1.0 | 1.0 | 2.0 | 1.0 | 1.0 |

Discussion.—This bit of evidence cannot be taken with any degree of finality, but it indicates that polarity, as it influences the formation of callus, may not be dependent on a simple food relation; it suggests that the polarity may be based on a complex of factors imperfectly understood at the present time. Kostoff (1928), working with whip grafts of solanaceous plants, found that starch accumulates just above the callus, and stated that "this great accumulation of food is the specific cause of the proliferations." While the dependence of callus development on the accumulation of food is an interesting possibility, it has not been satisfactorily demonstrated as yet, so far as the writer knows.

Since these studies have pertained particularly to root-grafts in which the top of the root-piece is united with the bottom of the scion-piece, nearly

all measurements of root callusing have been of callus formed from the top ends of the root-pieces. Many observations indicate that the root calluses practically as well as the scion (though root callusing may not be so uniform, owing to inherited differences because of seedling origin), but the top end of the root neither calluses as well as the bottom end of the scion nor as well as the bottom end of the root.

Significance of Downward Polarity in the Formation of Callus Overgrowths

The preceding experiments have shown the distinct characteristic of apple scion and root cuttings to form callus tissue more abundantly from the lower ends. This fact seems to have a direct relation to the usual occurrence of graft-union overgrowths as proliferations of the scion (particularly the lip) rather than of the root stock, and further supports the view that overgrowths found at the unions of young apple trees are frequently of callus derivation, their formation being very largely influenced by this decided polarity. Plate XXII, figure 1 shows Wealthy grafts with overgrowths of scion derivation at the unions as well as those of stock origin at the bottom of the root pieces.

Effect of Variety on Callus Formation

Observations on the callusing of cuttings of several apple varieties have repeatedly shown that varietal differences exist both in the rate of forming callus and in the final abundance attained under like external conditions. Since nurserymen find some varieties more susceptible to "callus knot" than others, a demonstrated correlation between varietal callusing capacity and varietal susceptibility to overgrowths may be regarded as of particular interest.

Experiment 14 (Variety)

Methods.—See experiment 6. Any procedure that permits the normal formation of callus serves to illustrate the importance of variety.

Results.—Table 17 shows that different varieties vary markedly in the

TABLE 17. *Comparative Amounts of Callus from Scion Cuttings of Five Varieties*

| Variety | Total Callus in Millimeters | | |
|-------------------------|-----------------------------|---------|---------|
| | 7 Days | 16 Days | 21 Days |
| Yellow Transparent..... | 22.25 | 63.75 | 78.50 |
| Wolf River..... | 27.50 | 63.00 | 71.25 |
| Ben Davis..... | 24.25 | 61.50 | 62.75 |
| Delicious..... | 15.25 | 34.50 | 37.75 |
| Willow Twig..... | 10.00 | 35.50 | 49.75 |

rate at which they form callus. These data indicate that Yellow Transparent, Wolf River, and Ben Davis are rapid callusing varieties, whereas the Delicious and Willow Twig are comparatively slow callusing varieties.

Other observations have indicated that in general the varieties that

exhibit the highest initial rates of callus formation are also the ones that develop the largest final volumes before callus growth ceases. The Yellow Transparent was always found to be a prolific callusing variety. In many experiments this variety was used in conjunction with the Wealthy and Jonathan varieties, and in these experiments the Yellow Transparent usually produced the most callus, the Wealthy less, and the Jonathan least. This is shown in tables 18 and 19. Differences between varieties in abun-

TABLE 18. *Comparative Amounts of Callus from Cuttings and Grafts of Three Varieties*

| Variety | Total Callus in Millimeters | |
|-------------------------|-----------------------------|---------|
| | 21 Days | 52 Days |
| Yellow Transparent..... | 70.0 | 132.0 |
| Wealthy..... | 66.0 | 126.0 |
| Jonathan..... | 58.0 | 112.7 |

TABLE 19. *Comparative Amounts of Callus from Cuttings and Grafts of Two Varieties*

| Variety | Total Callus in Millimeters | |
|-------------------------|-----------------------------|---------|
| | 18 Days | 50 Days |
| Yellow Transparent..... | 76.0 | 141.0 |
| Jonathan..... | 52.0 | 114.0 |

dance of callusing are more marked in some instances than in others, but consistent differences usually exist.

Discussion.—It is a very significant fact that the abundant-callusing varieties have generally been found to be particularly subject to union overgrowths. Thus, the Yellow Transparent and Wealthy varieties knot badly as compared with many other varieties. In a study of overgrowths Muncie (1926) frequently used the Wealthy variety because of the high percentage of trees of this variety which were discarded in the nursery on account of overgrowths at the graft union. If a large number of commercially grown apple varieties were arranged in a series according to the abundance with which their cuttings formed callus under similar conditions, it seems very probable that this series would agree closely with a series based upon susceptibility to callus knot in the field.

GENERAL DISCUSSION OF RESULTS

Just as suitable conditions of heat, moisture, and oxygen are essential for the growth of all plants and animals, so are they essential for the growth of callus cells. Published information is very limited with reference to the influence of temperature upon callus development. This is especially true with respect to the callusing of stem and root cuttings of apple. There has been an equal lack of information as to the effects of moisture, as well as of other environmental factors, on callus formation. The present study was begun with a view of gaining more definite information on some of these points. It was thought that it would yield data which, in addition to being of general scientific value, might be useful in apple root-grafting practices, applying not only to the formation of wound tissue but to the prevention of excessive callusing.

The results show that temperature may be employed as an effective instrument in the control of callus formation. Within a wide temperature range, higher temperatures greatly accelerate callus formation. A temperature between 0° and 5° C. lies close to the lower limit of this range, and allows only a small amount of callus to be formed after a period of several months. At 10° C. callus formation is more rapid than at 5° , and at 15° it proceeds at a still higher rate. Growth curves within this range are uniformly concave (indicating an acceleration with the lapse of time), whereas curves for temperatures ranging from 20° upwards are convex (showing rapid growth of callus during the early part of the storage period, but a gradual retardation later). Although the rate of callus formation increases with rise in temperature, if the temperature is above 32° C. pronounced injury occurs. Above this point browning of the surface cells takes place, and the injured cells are readily attacked by molds. By the proper manipulation of the temperature a desired degree of callusing may be had in a given length of time. The temperature can be so regulated that apple grafts can be callused to a point where they are ready for setting out within several days' time, or by using a lower temperature the time for reaching this abundance of callusing may be extended over several months. Or the grafts may be properly callused and then kept at a sufficiently low temperature to prevent further bud, root, and callus development until such a time as they can be planted.

The moisture conditions of the environment have a pronounced retarding influence on callus formation when they are such as to allow considerable desiccation of the tissues. The percentage relative humidity of a surrounding atmosphere seems to be of less importance than the continuous availability of liquid water to the plant material. Cuttings that were exposed to 100 percent relative humidity usually callused only slightly; but if the cuttings were completely covered with a moist medium of peat moss, sphagnum moss, or sand, so that a film of water was held against their surfaces, they callused abundantly. Some callus will form in atmospheres of less than 100 percent relative humidity, but it is usually meager in amount and short-lived. Once saturation of the atmosphere has been attained and a film of liquid moisture has been made available to the plant materials, little or no stimulation of callus development results from increasing the moisture content of the surrounding medium. It was found that with the type of peat moss used, 100 percent water (with respect to dry weight of the peat moss) was sufficient to permit good callusing, and that the water content of the medium may be increased several times (to 300-400 percent) with no great modification of result. Peat moss was found to be a very satisfactory medium for experiments on callusing because of its great absorptive and water-holding capacity. If cuttings or grafts are covered deeply with peat moss containing 300 percent water, no further water need be added under ordinary conditions for a month or more.

Sand is perhaps the most commonly employed medium for callusing of cuttings and grafts, but it differs greatly from peat moss in its physical properties. When water is added to sand the spaces between the particles are flooded (absorption is negligible), but this water is held very loosely due to the coarseness of the medium and to its inability to absorb. In a warm, dry room water must frequently be added to the medium. The alternate flooding and drying of the medium does not favor callus formation, and unsatisfactory results may be obtained.

Oxygen is essential for callus formation, but a lower percentage than that of ordinary air is sufficient. Callus development is not checked by extremely high concentrations of oxygen. Evidence was obtained which indicates that high concentrations of carbon dioxide inhibit callusing.

Apple cuttings, whether shoot or root in derivation, manifest a distinct basal polarity in the formation of callus. That is, the lower end of both scion and root cuttings calluses with distinctly greater abundance than the upper end. If a slanting cut be made on the basal end of a scion cutting, the lower end, or lip, of this cut surface produces far more callus than the upper end. The top end of either a scion or a root cutting calluses comparatively poorly, regardless of whether the cut is transverse or slanting. The explanation of this polarity is not known. It does not appear to be an effect of gravity, unless gravity influences the cuttings before they were severed from the parent tree. The cuttings manifest the same polarity whether stored for callusing in an upright, a horizontal, or an inverted position. Although preliminary tests have not been successful in showing a dependence of polarity upon food storage, this is a possibility. Root knot or callus overgrowths are doubtless related to polarity in callus formation, since in most cases these are outgrowths from the scion lips of root-grafted trees. Since in a tongue-graft union the scion lip occurs on the bottom end and the root lip occurs on the top end of the cutting, it is the scion lip which calluses the more abundantly. With suitable temperature and moisture conditions and a continuous supply of descending elaborated food from the growing tree, it seems very probable that the original callus roll from the scion lip might continue its growth over several years.

Cuttings from selected varieties differed as to the rate and final abundance of callus formation. The Yellow Transparent, Wealthy, Wolf River, and Ben Davis varieties have been found to be abundant callusers, whereas the Jonathan, Delicious, and Willow Twig varieties produce callus less vigorously. These differences may have a direct relation to varietal susceptibility to overgrowths at the graft union, since the abundant-callusing varieties are particularly subject to proliferations of this type.

SUMMARY

Recent investigations on the crown gall disease have indicated that a large proportion of the overgrowths on root-grafted apple trees are in the

nature of callus rather than bacterial tumors. Thus the necessity arose for studying the influence of such important environmental conditions as temperature, moisture, and aëration on the callusing of apple cuttings and grafts. Both constant temperatures (ranging from 0° to 40° C.) and variable temperatures were used. Variable moistures were provided in two ways: (1) by varying the humidity of the atmospheres used, and (2) by varying the water content of a peat moss callusing medium. Aëration conditions included atmospheres of different oxygen, nitrogen, and carbon dioxide concentrations. During the course of the study repeated observations have also been made on polarity phenomena and varietal differences which seemed pertinent to the formation of callus overgrowths. The results may be summarized as follows:

1. The complete range of temperatures permitting the formation of callus from apple cuttings (scion or root) and grafts was found to lie between 0° and 40° C. At 3°-5° only a small amount of callus developed during a period of several months. Between 5° and 32° the rate of callus formation increased and the time elapsing before attainment of final volume decreased with rise in temperature. At temperatures above 32° injury usually resulted, and at 40° death of the tissues, accompanied by mold formation, always occurred within the first few days.

2. By the proper regulation of controlled temperatures the callusing processes may be so accelerated or retarded that, within reasonable limits, a desired degree of callus formation may be had within a given length of time. Hence, apple grafts may be callused over a period of several months; they may be similarly callused within a few days, or, after being properly callused, they may be held in good condition for at least several months before planting.

3. For general callusing purposes, temperatures below 20° C. rather than higher have been found most satisfactory.

4. Variable temperatures do not change the general relations. Callusing is accelerated or retarded according to the degree and duration of the temperature.

5. Air moistures below saturation have generally been found to be inhibiting in their effect on callus formation, since below this point desiccation of the tissues occurs. As the moisture content of the air falls, the rate of desiccation increases.

6. Liquid water, present as a film inclosing the cutting, appears to provide the most favorable moisture conditions for bringing about uniform callusing. Such conditions are supplied by moderately moist peat, sphagnum, or sand.

7. Good callusing takes place in a peat moss medium containing 100 percent water by weight. Practically no increase is brought about by raising the water content of the medium, and no perceptible injury or inhibitory effect occurs if the proportion of water to oven-dried peat moss

is increased from three to four times. With a water content beyond this point, however, aëration probably becomes a limiting factor, and callusing is inhibited.

8. Desiccation of callus tissue is accelerated by increase in temperature and decrease in humidity.

9. Proper aëration was found to be important for callusing. The evidence indicates that while some oxygen is required, an amount of oxygen below that of air (20 percent) is sufficient. Callusing took place in high concentrations of oxygen, but was inhibited in 100 percent oxygen. High concentrations of carbon dioxide, particularly with a limited supply of oxygen, prevented callusing.

10. Both scion and root cuttings of apple manifested a distinct polarity in callus formation. Dominance of the bottom end over the top end of cuttings held regardless of the position of the cuttings, whether upright, horizontal, or inverted.

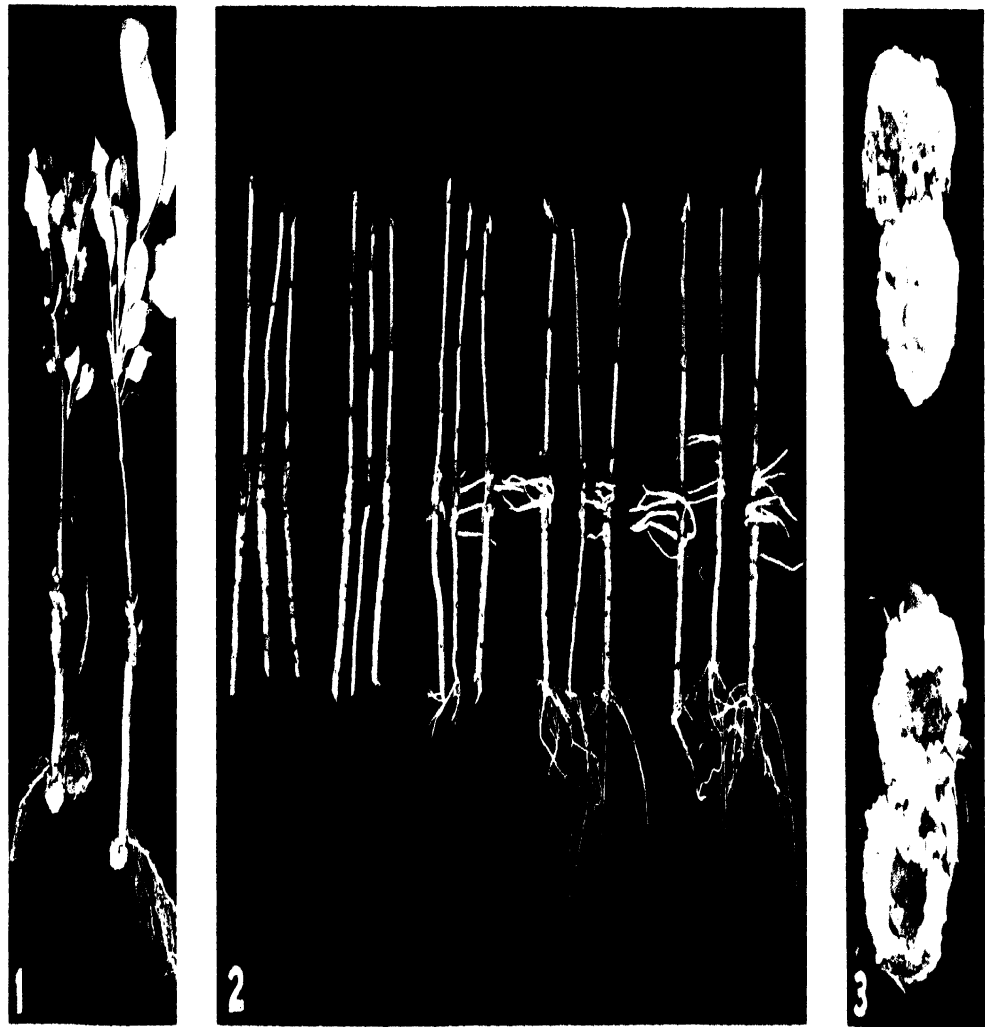
11. Varieties differ both in the rate and abundance with which they form callus. The Yellow Transparent, Wolf River, Wealthy, and Ben Davis are abundant callusing varieties, and the Jonathan, Delicious, and Willow Twig are moderate callusing varieties.

12. The effects of polarity and varietal differences on callus formation of apple cuttings and grafts appear to be significantly related to the occurrence of overgrowths at the union of root-grafted trees.

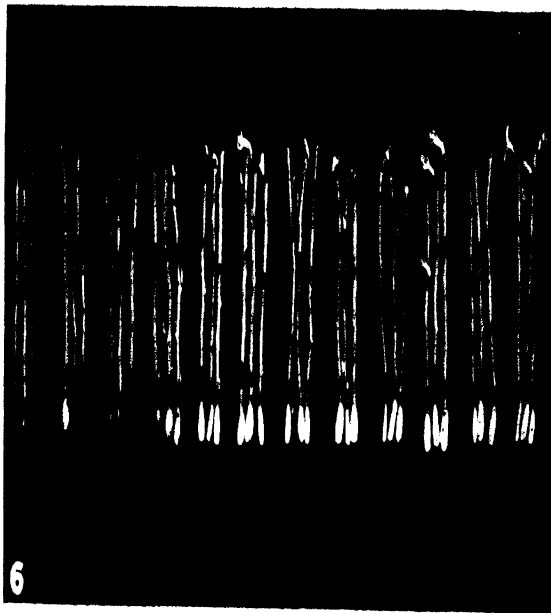
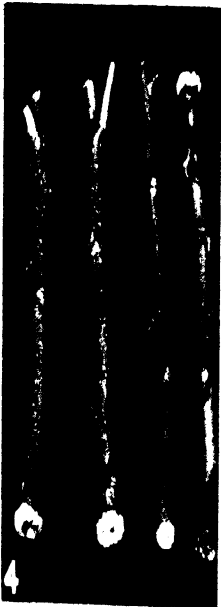
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SHIPPY; CALLUS



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EXPLANATION OF PLATES

PLATE XXII

FIG. 1. Apple grafts (Wealthy × French crab) after growing 86 days in the greenhouse. The graft on the left shows a slight overgrowth from the scion lip, and both grafts show distinct overgrowths from the bottom ends of the root pieces.

FIG. 2. Apple grafts (Wealthy × French crab) which have been stored in moist peat moss at different temperatures. Typical shoot and root development is shown for different storage temperatures and times. The callusing of the grafts has proceeded nearly parallel with these other growth activities. Reading from left to right: 5° C. for 48 days, 10° for 40 days, 10° for 48 days, 15° for 40 days, and 15° for 48 days.

FIG. 3. Abundant callus formation from the bottom ends of apple cuttings, as follows: upper, Delicious cuttings; lower, French crab root cuttings. The calluses in both cases have grown together.

PLATE XXIII

FIGS. 1-5. Jonathan cuttings which were callused at similar temperatures in peat moss which varied greatly in water content. The several figures combined illustrate the broad moisture tolerance manifested by callusing tissues. Average moisture contents of the peat moss media (percentage water by weight per 100 parts oven-dried peat moss) for the entire storage period were as follows: reading left to right: 97, 177, 271, 337, and 437 percent. In addition, the effect of polarity on callusing is shown by the unequal ability of the two ends of the same cutting to form callus.

FIG. 6. The figure shows the injury which resulted in high concentrations of oxygen when Wealthy cuttings were stored for 34 days in various mixtures of oxygen and nitrogen. Since callusing of the cuttings was negligible during exposure to the gas, they were transferred to optimum conditions (moist peat at a moderate temperature) in order to test injury. The result is shown after 14 days. The initial gas mixtures for the different sets of three cuttings each were as follows, reading right to left: air check, 0 percent oxygen (nitrogen used as complementary gas in each case), 10 percent oxygen, 20, 30, 40, 50, 60, 70, 80, 90, and 100 percent.

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A TAXONOMIC STUDY OF SPECIES OF THE GENUS *VAUCHERIA* COLLECTED IN CALIFORNIA

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INTRODUCTION

The genus *Vaucheria* is one of the best known and most widely distributed members of the Chlorophyceae. Its frequent occurrence, both on damp ground and in shallow ponds and streams, its conspicuous size and unusual reproductive structures account, no doubt, for the extensive taxonomic study that has been given it for over a century. The genus is so familiar to botanists that it is unnecessary to present a discussion of its generic characteristics and modes of reproduction. As is also well-known, the differentiation of species is based almost entirely upon the structure of the reproductive organs, a system initiated by Vaucher (1803).

European botanists have devoted considerable attention to systematic studies of the genus, the best recent treatment appearing in the volume by Heering (1921) on the Siphonales and Siphonocladiales in Pascher's well-known series. While species of *Vaucheria* have been frequently collected and identified by American botanists, and are included in lists of algae reported from many parts of the country by Transeau, Tiffany, Setchell and Gardner, and Taylor, the only taxonomic treatments of all American species are those of Wolle (1887) and of Collins (1909). The recent monographic treatment of the Vaucheriaceae by Miss Brown (1929) is largely a compilation from the works of Heering (1921) and of Collins (1909), but completely ignores the logical division of the genus into sections as presented by these and other authorities.

Since the various American workers on the group are in marked disagreement as to the exact nature of the specific limits of representatives of the genus found in this country, it was suggested to the writer by Dr. G. M. Smith that a careful study of a large number of collections might give a more clear-cut conception of the various species, and in addition might reveal members of the genus not previously reported from America.

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The data presented herewith are based upon an extensive series of collections of aquatic and terrestrial species of *Vaucheria* which were gathered from a large number of stations throughout central California. Since a comparatively large area was rather thoroughly covered, and since collections were made throughout an entire year, the writer feels that the present paper gives (with one notable exception to be mentioned later) a rather complete account of the fresh water species within the region studied.

At the outset of the study, no attention was given to the *Vaucherias* of marine and brackish waters, since Setchell and Gardner (1919) report that material from such stations is always sterile. However, during the summer of 1929 fruiting material of a marine species was discovered at Elkhorn Slough, twenty miles north of the Hopkins Marine Station at Pacific Grove, by Dr. G. M. Smith and Dr. I. F. Lewis. A study of it has been included in this paper.

Comparative studies of individuals of the same species from different stations are based upon both direct microscopical observation and an extensive series of accurate camera lucida drawings. By making all drawings of the same magnification, it is possible to obtain comparisons of shape and size of fruiting organs, and to determine which morphological characteristics are constant and which are variable in nature. The dimensions recorded in this paper are based upon measurements made in connection with the study of each species. However, the writer feels that little emphasis should be placed upon size in the determination of species in this genus, and the marked disagreement of different authorities as to the dimensions of filaments and zygotes is not a matter of vital importance. The characteristics of the reproductive structures of the species here described are so striking as to make accurate identification possible without strict adherence to a set of measurements. In several cases, the dimensions of filaments and zygotes do not correspond to those given in the original descriptions; yet the form of the antherids and oögones makes identification certain. Measurement for size of fruiting material may be based either on the dimensions of the zygote or of the oögone. The former method, which is followed by Heering (1921), is not as satisfactory as the latter which is followed by Collins (1909), because the collections may consist of material with immature zygotes, or there may be distortion of the zygote wall due to plasmolysis.

It has seemed desirable to include descriptions and figures of such well-known species as *V. sessilis* and *V. geminata* to emphasize specific characters of the less common forms. This study has resulted in a more conservative treatment than that given by Collins (1909), and an agreement, in most cases, with the specific limits set by Heering. The prophecy of Tiffany (1926) that further study would reveal additional intergrades between certain species and varieties of Heering (1921) (notably *V. geminata*) has

not been fulfilled in all cases, since there appear to be constant morphological differences.

Most taxonomic treatments of the genus group species into certain sections which are founded upon the structure of the antherid. These structural features are its shape, manner of opening, and the presence or absence of a special supporting cell which separates it from the remainder of the filament. On this basis Heering (1921) lists seven sections. The antherid of those species included in the sections *Androphorae* and *Piloboloideae* has a definite supporting cell. All species included in these sections are marine or brackish water forms. The marine form included in this paper belongs in the *Piloboloideae*. Species of the remaining five sections—*Woroninia*, *Tubuligerae*, *Globiferae*, *Corniculatae*, and *Anomalae*—lack a supporting cell. Members of the section *Woroninia* are reported only from marine or brackish water habitats. The fresh water species are placed in the remaining four sections and representatives of each of these have been collected from California. *V. aversa*, the only species of the section *Tubuligerae* reported from California by Collins, was not found in any of the collections studied and has, for that reason, been omitted from this paper.

DESCRIPTIONS OF THE SECTIONS

The six sections represented in the local flora may be distinguished as follows:

SECTION PILOBOLOIDEAE Walz, Nordstedt emend.

Plants monoecious or dioecious. Antherid separated from the filament by an empty supporting cell; cylindrical, bearing one to several short lateral projections, each opening by a round terminal pore. The oögone sessile on the filament or borne at the end of a lateral branch; in one species separated from the branch by an empty supporting cell. Zygote spherical, not completely filling the oögone.

SECTION TUBULIGERAE Walz

Antherid sessile on the filament, straight or but slightly bent, cylindrical in shape, opening by a more or less elongate slit beginning at the apex. Oögone likewise sessile, or very short-stalked, obliquely ovoid, the zygote ovoid and not completely filling the oögone. Mature zygote with a three-layered wall and a distinctly reddish color.

SECTION GLOBIFERAE Heidinger

Antherid borne at the end of a more or less curved stalk, distinctly saccate or globose. Oögone sessile, spherical, its wall distinctly spotted, the zygote completely filling the oögone and with a thick many-layered wall at maturity.

SECTION CORNICULATAE Walz

Antherid borne at the end of a more or less bent stalk, tubular, hooked to spirally coiled, opening by a single round terminal pore. Oögones

either sessile or pedicellate, spherical to convex-concave. Species of this section are grouped into three sub-sections as follows:

SUB-SECTION *Sessiles* Walz

Oögones sessile on the vegetative filaments, one or two adjacent to an antherid; the oögone with its major axis perpendicular, parallel or oblique to the filament and having a distinct lateral or terminal beak.

SUB-SECTION *Racemosae* Walz

Oögones distinctly pedicellate, one to several borne on a lateral fruiting branch terminating in an antherid. Length of the fruiting branch and of pedicels, and number of oögones, often variable for a particular species.

SUB-SECTION *Radiatae* New Sub-section

Oögones and antherids borne on unusually long, slender pedicels; 2-12 pedicels arising from a restricted region on the filament which may or may not be slightly elevated; those bearing antherids more or less perpendicular to the filament, and those bearing oögones at an angle of about forty-five degrees to it. Differing from other members of the *Corniculatae* in the frequent occurrence of more than one antherid with an oögone or group of oögones.

SECTION ANOMALAE Hansgirg

Distal end of the antherid much broader than the basal portion; antherid opening by one to several definite lateral pores. Oögones pedicellate, one to three or four borne on a lateral fruiting branch terminating in an antherid.

One of the striking features of the American literature on *Vaucheria* is the lack of records and descriptions of species of the section *Anomalae*. It is quite possible that the antheridial characteristics of these species have not been sufficiently appreciated by botanists of this country, and the absence of definite records may be accounted for in this manner. Both species included in the *Anomalae* were found during the course of this study, and the figures, measurements, and descriptions presented are of this material.

DESCRIPTIONS OF THE SPECIES

SECTION PILOBOLOIDEAE

VAUCHERIA LONGICAULIS sp. nov.

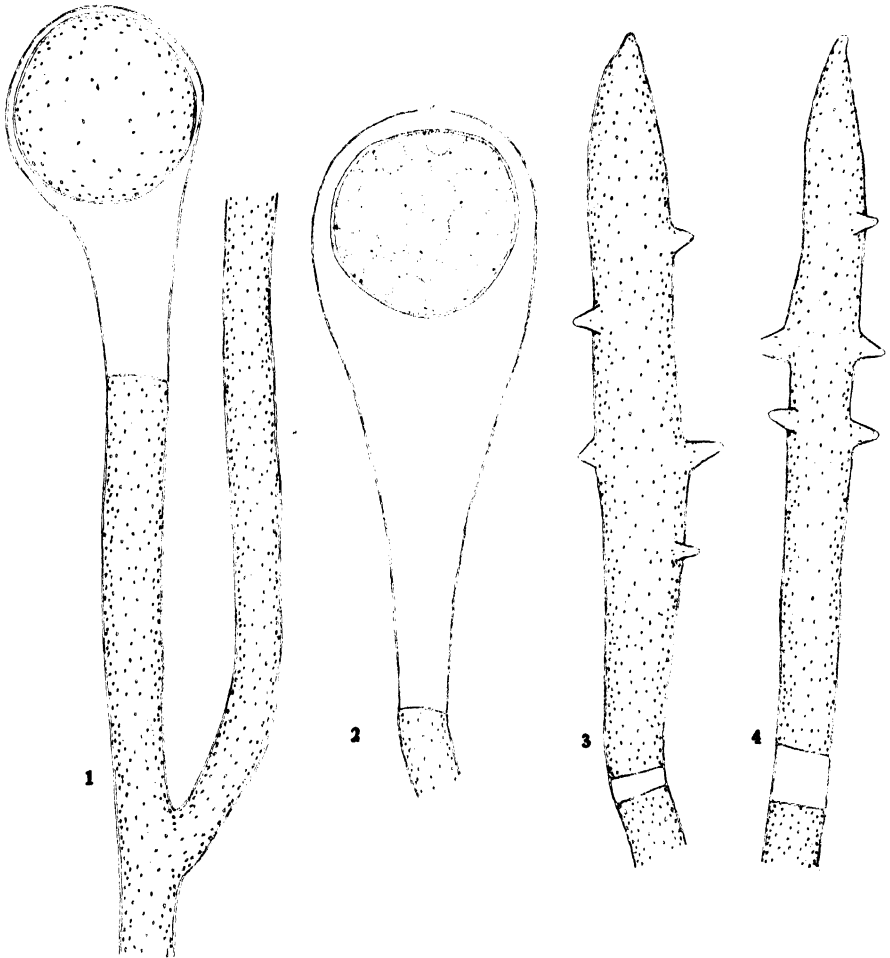
Text figures 1-4

Plants dioecious. Male plants with antherids separated from the filament by an empty supporting cell; antherids cylindrical, bearing from three to twelve (regularly five to eight) short lateral projections, each terminating in a round pore. Female plants with oögones borne at the

end of more or less elongate lateral branches; oögones erect, club-shaped, the distal portion becoming gelatinized at maturity. Zygote sub-spherical and only occupying the upper portion of the oögone. Width of filaments, 33-60 μ . Oögones, 110-165 μ wide; 275-440 μ long. Antherids, 330-730 μ long; 45-60 μ wide.

Collected on mud flats exposed at low tide in Elkhorn Slough near Castroville, Monterey County.

This species resembles *V. litorea* Agardh in many respects, and is undoubtedly a closely related form. Nordstedt (1879) and Farlow (1881),



TEXT FIG. 1. *V. longicaulis*. Portion of female plant bearing immature oögone. TEXT FIG. 2. *V. longicaulis*. Mature oögone. TEXT FIGS. 3 and 4. *V. longicaulis*. Mature antherids.

who have studied this species most thoroughly, both emphasize the presence of an empty supporting cell between the oögone and the filament. There was never such a definite cell in any of the material from Elkhorn Slough.

*V. longicaulis** also differs from *V. litorea* in having much longer oogonial branches and in having these branches erect instead of reflexed. Two to four lateral projections, opening by terminal pores, are reported for *V. litorea*. Most individuals of *V. longicaulis* have antherids with 5 to 8 projections, and there may be as many as twelve. These striking differences seem to warrant recognition of the Pacific Coast material as distinct from *V. litorea*.

SECTION GLOBIFERAE

VAUCHERIA PACHYDERMA Walz

Plate XXIV, figures 6 and 7

Walz, 1866, p. 146, Pl. 12, figs. 1-6; Heering, 1907, p. 151, fig. 74; 1921, p. 86, fig. 73; Heidinger, 1908, p. 318, Pl. 19, figs. 1-5; Brown, 1929, p. 94, Pl. 15, fig. 9.

V. Dillwynii (Web. and Mohr) Agardh, 1810, p. 21; Collins, 1909, p. 423.

Oögone sessile, regularly solitary, the lateral beak parallel to the filament; wall finely spotted. Zygote but little broader than long, when mature with a thick wall composed of several definitely distinguishable layers (supposedly seven). Antherids solitary, adjacent to the oögone, terminating a stalk usually bent at ninety degrees to the major axis of the oögone. Antherid distinctly saccate, much broader than the stalk.

Width of filaments 50-70 μ . Oögones 98-120 μ broad, 90-100 μ long.

Previously reported from Iowa, Massachusetts, Michigan, Minnesota, and New Jersey. There is no previous record of the species from California.

V. pachyderma is a relatively rare species, occurring in but three of the collections made. It was found growing in a dense mat on damp soil near the Administration building at Stanford University. Abundantly fruiting material was collected from a shallow pool on Sky-line Drive west of Palo Alto, and from a stream on the Overlook Road west of Los Gatos. A fourth station was reported by Dr. Smith in pools formed by the overflow of the lake on the Stanford campus, but no material from this station was studied.

As has been previously noted, the character which distinguishes this species from those of the Corniculatae-Sessiles group, and especially *V. borealis* Hirn, is the distinctly saccate nature of the antherid, a feature which is evident even in very immature material.

Collins (1909) applies the name *Dillwynii* to this species, using a name given by Agardh (1810) to a *Vaucheria* which may have been the one of the Collins description. None of the early descriptions or figures make any note of the nature of the antherid, the distinctive character of this species. Walz (1866) was the first to describe and figure this *Vaucheria* in such a way that it could be identified as a distinct species. For this reason it seems best to follow Heering (1921) in using the specific name given by Walz.

Miss Brown (1929) fails to recognize that *V. Dillwynii* (Web. and Mohr) Agardh of Collins (1909) is synonymous with *V. pachyderma* Walz. It seems important to note that the species is previously reported from this country, and is not strictly limited to Europe, as her paper indicates.

SECTION CORNICULATAE

SUB-SECTION SESSILES

VAUCHERIA SESSILIS (Vauch.) De Candolle

Plate XXIV, figure 1

De Candolle, 1805, p. 63; Hansgirg, 1886, p. 94; Wolle, 1887, p. 151, Pl. 127, figs. 9-11; Goetz, 1897, p. 111, figs. 17-22; Heering, 1907, p. 146, fig. 69; 1921, p. 87, fig. 74; Collins, 1909, p. 425; Brown, 1929, p. 93, Pl. 15, fig. 11.

Oögones sessile, generally two, less often one, having the long axis at an angle to the filament, and a short oblique beak. Mature zygote completely filling the oögone, having distinct dark spots and a three-layered wall. Antherid solitary, usually between two oögones, on a short, more or less bent stalk; the antherid of the same diameter as the stalk and opening by a terminal pore.

Width of filaments 60-110 μ . Oögone 60-80 μ wide, 80-98 μ long.

Previous records show a wide distribution through the United States, the species having been reported from the following states: California, Connecticut, Illinois, Iowa, Maine, Massachusetts, Michigan, Minnesota, Nebraska, New Jersey, Rhode Island, and Washington.

V. sessilis is one of the best known and most widely distributed species of *Vaucheria*, occurring in nearly a third of the collections made. It is, as a rule, aquatic, usually growing in slowly moving streams or forming extensive mats in permanent shallow pools. However, at one station, a stream at the side of the coast road two miles north of Santa Cruz, it was collected on damp sand at the edge of the water.

Three species, very closely related to *V. sessilis* (Vauch.) DC. as described above, have been recognized by certain workers: *V. repens* (Hassall) Hansgirg, with its beak parallel to the filament; *V. clavata* (Vauch.) DC., with a beak perpendicular to the filament; and *V. orthocarpa* Reinsch, resembling *V. clavata* except for a thick seven-layered zygote wall. Heering (1907) has shown that the position of the beak is not a sufficiently marked character to warrant specific rank, and has reduced them to forms of *V. sessilis*. The material studied gives evidence for following his interpretation, at least in so far as *V. repens* and *V. clavata* are concerned.

VAUCHERIA SESSILIS forma REPENS (Hassall) Hansgirg

Plate XXIV, figure 2

Hansgirg, 1866, p. 94; Heering, 1907, p. 144; 1921, p. 88, fig. 75; Brown, 1929, p. 94, Pl. 15, fig. 8.

V. repens Hassall, 1843, p. 430; 1845, p. 52, Pl. 6, fig. 7; Rabenhorst, 1868, p. 267; Goetz, 1897, p. 110, figs. 14-16; Hirn, 1900, p. 86, fig. 1; Collins, 1909, p. 425.

V. sessilis variety *repens* Tiffany, 1926, p. 77.

Oögone sessile, usually solitary, having the long axis at an angle to the filament and the beak parallel to it. Ripe zygote and antherid like *V. sessilis*.

Width of filaments 48-65 μ . Oögones 60-70 μ long, 50-70 μ broad.

Previously reported from California, Utah, and Iowa.

This form is not of such common occurrence as *V. sessilis*, and has been found in but two of the collections made. The alga was terrestrial in both stations and was found on damp shaded ground at the end of Felt Lake near Stanford University, and on damp sand at the edge of the Soquel river in Santa Cruz county. In habitat, then, it seems to differ from *V. sessilis*, which is only rarely terrestrial.

The vegetative filaments of this form are narrower than those of *V. sessilis*. However, in this, as in other Vaucherias, size of filaments is always too variable a character to be of any real significance. The parallel beak of the oögone is, as a rule, quite striking, but as has already been stated, does not seem sufficiently distinctive to give this form specific rank.

VAUCHERIA SESSILIS forma CLAVATA (Vauch.) Heering

Plate XXIV, figures 3 and 4

Heering, 1907, p. 147, fig. 71; 1921, p. 88, fig. 76; Brown, 1929, p. 93, Pl. 18, figs. 22-23.

V. clavata (Vauch.) DC. Klebs, 1892, p. 70; 1896, p. 94, fig. 2; Goetz, 1897, p. 114, figs. 23-28.

V. orthocarpa Collins (non Reinsch), 1909, p. 427.

Oögone sessile, solitary, or two with an antherid between, having the long axis perpendicular to the filament and the beak apical. Mature zygote with a central red spot and a three-layered wall. Antherid like that of *V. sessilis*.

Width of filaments 49-78 μ . Oögone 50-75 μ wide, 78-99 μ long.

Previously reported from California, Michigan (?), and Iowa (?).

The only specimens available for study were in the form of prepared slides made by Dr. D. A. Johansen. The material was collected locally, but the exact station is unknown. No other collections of the *V. sessilis* type contained oögones with the distinctly apical beak and bilateral symmetry characteristic of this form. While the beak of the oögone was

usually perpendicular to the filament, there were frequent instances in which it was more or less oblique, tending to resemble the characteristic position of the beak of *V. sessilis* (Pl. XXIV, fig. 3).

Vaucher's description and figure of *Ectosperma clavata* indicate that he observed only the zoösporangies of what might have been almost any species of *Vaucheria*, and the same is true of the figures and description of Hassall (1845). The first account of the reproductive organs is given by Klebs (1896), who unfortunately revived the old Vaucherian name, and it is upon this conception of the species that the descriptions of Goetz (1897) and Heering (1921) are based.

As has been previously stated, *Vaucheria orthocarpa* Reinsch (*Vaucheria sessilis* forma *orthocarpa* (Reinsch) Heering) differs from *Vaucheria sessilis* forma *clavata* (Vauch.) Heering only in the thick, seven-layered wall of the mature zygote. This, however, is a definite character and one easily recognized.

There is a distinct tendency throughout the literature to confuse *V. orthocarpa* and *V. clavata*. Collins (1909) reports *V. orthocarpa* Reinsch from California, but his description of the alga includes the note that the wall of the zygote is three-layered. From this it may be concluded that his species is not based on the original description of Reinsch, but rather upon the description of *V. clavata* given by Goetz (1897) who considers *V. orthocarpa* synonymous with *V. clavata*. It follows that the *V. orthocarpa* reported from California by Collins is, in reality, *V. sessilis* forma *clavata*. The material studied is also this form, for it shows no evidence of the unusually thick, seven-layered wall typical of the form *orthocarpa*. It is possible, also, that Transeau's record of *V. orthocarpa* from Michigan and that of Tiffany of *V. sessilis* forma *orthocarpa* from Iowa are based on the description of Collins rather than that of Reinsch or Heering, although this cannot be determined, since there are no descriptions nor figures in either case.

Miss Brown (1929), while following the interpretation of *clavata* as a form of *V. sessilis*, as presented by Heering (1921), describes *V. orthocarpa* Reinsch as a distinct species, including the note that the zygote wall is three-layered. She offers no explanation for this apparent inconsistency, nor for ignoring what seems to be an important feature of the original description.

VAUCHERIA BOREALIS Hirn

Plate XXIV, figure 5

Hirn, 1900, p. 87, fig. 2; Heering, 1907, p. 150, fig. 73; 1921, p. 89, fig. 78; Norrington, 1927, p. 288; Taylor, 1928, p. 108, Pl. 13, figs. 21-22; Brown, 1929, p. 94, Pl. 16, fig. 11.

Oögone sessile or very short-stalked; regularly solitary; long axis parallel to the filament, beak horizontal. Mature zygote with an unusually thick,

three-layered wall. Antherid single, adjacent to an oögone, terminating a more or less bent stalk, hooked or coiled, opening by a terminal pore.

Width of filaments 45–80 μ . Oögones 120–145 μ long, 90–105 μ broad.

Previously reported from Utah by Miss Norrington.

Two collections, one from a pool near Milpetas, and the other from damp soil at Moss Beach, San Mateo county, contained a *Vaucheria* agreeing closely with the original description of *V. borealis* Hirn (1900) except for the somewhat small size of filaments and oögones.

The structure of the oögone, with its long axis parallel to the filament and its distinctly horizontal beak, is a sufficiently striking character to distinguish this species from *V. sessilis*. Although the shape of the immature oögone is similar to that of *V. pachyderma*, the oögone of the latter species, as noted above, has a distinctly spotted wall. *V. borealis* may also be distinguished from *V. pachyderma* by its Corniculate type of antherid.

The finding of this species at sea-level stations is of particular interest, since previous collections have come from northern Europe, usually in mountain regions, and from high elevations in the Canadian and Utah ranges of the Rocky mountains. The California collections indicate that it is not, after all, a strictly boreal type.

SUB-SECTION RACEMOSAE

VAUCHERIA GEMINATA (Vaucher) De Candolle

Plate XXV, figures 8 and 9

De Candolle, 1805, p. 62; Walz, 1866, p. 147, Pl. 12, figs. 7–11; Wolle, 1887, p. 151, Pl. 128, figs. 1–3; Collins, 1909, p. 427; Heering, 1907, p. 154, figs. 78, 79; 1921, p. 89, fig. 79; Brown, 1929, p. 96, Pl. 18, fig. 31.

V. racemosa Goetz, 1897, p. 124, figs. 43–44.

Fruiting organs borne on lateral branches of varying length. Antherid solitary, terminating the branch, more or less bent or coiled. Oögones two to six, spherical to sub-ellipsoidal, borne laterally below the antherid on short erect pedicels on the fruiting branch. Mature zygote brown spotted, with three-layered wall.

Width of filaments 55–110 μ . Oögones 70–110 μ long, 60–110 μ broad.

Previously reported from California, Colorado, Connecticut, Illinois, Iowa, Indiana, Maine, Massachusetts, Minnesota, Michigan, Nebraska, New Jersey, and Washington.

This species is an extremely common one, of even more frequent occurrence than *V. sessilis*. It is both aquatic and terrestrial in habitat, and fruits abundantly in quiet water, where it is often found with *V. sessilis*.

The Corniculate type of antherid, usually projecting rather far beyond the oögones, the comparatively short erect pedicels of the oögones, and,

to a lesser extent, the shape of the oögones, make this a distinct species, easy to determine, even in immature material.

Individuals of *V. geminata* whose fruiting stalks bear more than two oögones are treated as a distinct variety, *racemosa*, by Walz (1866). Collins (1909) follows Walz in this respect, but states (p. 427) that "plants representative of each can often be found in the same tuft; the variety seems more abundant than the type." The extensive series of collections made in connection with this study gives a basis for a decision as to whether the variety *racemosa* is worthy of recognition as such. In most of the material studied, careful disentangling of single filaments from the mass showed many stalks bearing four or even six oögones on the same filament and adjacent to fruiting stalks bearing only two. The number of oögones is obviously an extremely variable character, and not of sufficient value to use in distinguishing a distinct variety nor even a form. This is in accordance with the interpretation of Heering (1921).

Collins (1907, p. 201, Pl. 76, fig. 1) describes, from California material, a new species, *V. longipes*, differing from *V. geminata* only in the unusually long fruiting branch and the long pedicels of the oögones. It seems well to note here that there is a decided discrepancy between the Collins description and figure. He describes the fruiting branches as being from 1 to 6 mm. long, but figures a branch only 6.5 times as long as the filament is broad, or not more than .6 mm. Tiffany (1926) presents a figure of *V. longipes* with a fruiting branch even shorter than that of Collins, and with shorter pedicels, stating that "even *V. longipes* Collins does not, apparently, always have such long fruiting branches as shown in Pl. 12, fig. 134." The figure given by Taylor (1928, p. 108, Pl. 13, fig. 19-20) does not give any information as to the length of fruiting branch found in his material.

A *Vaucheria*, found in a collection from moist shaded ground at Moss Beach, would, if identification were based upon a cursory examination of a few filaments, be classed as *V. longipes* Collins. Many of the fruiting branches were unusually long, exceeding in length those of the Collins figure, but none were more than 1 mm. A careful study of long filaments bearing a large number of fruiting branches revealed, however, that this unusual length was not constant. Very short fruiting stalks, resembling in every respect those of typical *V. geminata*, could be found adjacent to long ones. In none of the material was length of branch a sufficiently constant character to warrant recognition even as a form.

This study suggests that perhaps, in accordance with the view of Tiffany (1926), length of pedicel and of fruiting branch is not a sufficiently constant character to justify recognition of *V. longipes* as a distinct species. However, without further information than that provided by Collins (1907) in the figure and description, and without further material, it is not possible to settle this question.

VAUCHERIA UNCINATA Kuetzing (Goetz emend.)

Plate XXVI, figure 14

Kuetzing, 1856, p. 21, Pl. 60, fig. 1; Walz, 1866, p. 149; Goetz, 1897, p. 122, figs. 38-41; Heering, 1907, p. 161, figs. 86-87; 1921, p. 90, fig. 82; Norrington, 1927, p. 288; Brown, 1929, p. 97, Pl. 17, fig. 24.

Antherid and oogones borne on a short lateral fruiting branch. Oogones two to six, on pedicels usually bent downward toward the filament, occasionally horizontal or turned upward; length of pedicel equal to that of oogone. Antherid terminating the fruiting branch, more or less coiled, the stalk sharply recurved just above the insertion of the pedicels. Wall of mature zygote three-layered.

Width of filaments 52-70 μ . Oogone 55-78 μ long; 50-72 μ wide.

Previously reported from Utah by Miss Norrington.

A collection from shallow water in an irrigation canal eight miles east of Surf contained a *Vaucheria* corresponding closely to the description of *V. uncinata* Kuetzing given by Goetz (1897). Two other collections, one from a shallow stream on the Alpine Drive above Los Altos and one from a stream near Los Banos, also approached most nearly the description of this species, but most of the fruiting branches were in such a condition of abnormal proliferation as to make accurate determination impossible.

Kuetzing's original description and figures, and the description of Walz (1866) do not give an accurate conception of this species, and the identification of the material has been based upon Goetz's interpretation of the Kuetzing species.

The distinctly long, downward-curving or horizontal pedicels of the oogones are in sharp contrast to the stubby erect pedicels of *V. geminata*, and a careful study revealed no evidence of intergrading. The oogone is usually spherical, although the side toward the antherid may occasionally show a distinct flattening. A third distinguishing character, figured by Goetz but not included in the description, is the abrupt recurving of the antheridial stalk just above the pedicels of the oogone.

Although this species is comparatively rare, occurring in but three of the collections made, it is difficult to account for but one previous report from the United States. It seems a distinct, easily identified species. However, it is quite possible that *V. geminata* variety *depressa* Transeau is identified in this work as *V. uncinata*. It is also possible that many workers identify any *Vaucheria* of the Racemose type with two or more oogones as *V. geminata*.

Collins reports *V. uncinata* in a list of flora from Massachusetts, but does not include the species among those in his later work (1909). Wolle also reports the species in a list of algae in the Bull. Torrey Bot. Club 6, 1877, but omits it from his "Fresh Water Algae" (1887).

VAUCHERIA TERRESTRIS (Vaucher) De Candolle

Plate XXV, figures 10 and 11

De Candolle, 1805, p. 62; Walz, 1866, p. 149, Pl. 13, figs. 18-19; Wolle, 1887, p. 153, Pl. 129, figs. 1-8; Goetz, 1897, p. 21, figs. 35-36; Heidinger, 1908, p. 342, Pl. 19, fig. 9; Heering, 1907, p. 160, fig. 85; 1921, p. 90, fig. 80; Collins, 1909, p. 426; Brown, 1929, p. 98, Pl. 18, fig. 30.

Antherid hooked or spirally rolled, terminating the lateral fruiting branch. Oögone solitary, lateral, on a very short pedicel, bent in the same plane as the antherid. Mature zygote with numerous brown spots and a three-layered wall, the middle layer thick and shining.

Filaments 38-190 μ wide. Oögones 70-160 μ broad, 80-165 μ long.

Previous records from California, Connecticut, Illinois, Iowa, Massachusetts, Nebraska, New Jersey, and Washington.

V. terrestris, although of less common occurrence than *V. geminata* and *V. sessilis*, is another of the common, widely distributed species, frequently collected from both terrestrial and aquatic habitats.

The solitary oögone, often so shortly pedicellate that it appears to be practically sessile on the fruiting branch, is the feature which best distinguishes this species from both *V. geminata* and *V. hamata*. Another characteristic feature is the parallel bending of antherid and oögone. The fact that the oögonial wall adheres to the mature zygote when it breaks away from the pedicel, and later gelatinizes and disappears, is a point particularly emphasized by certain workers. Since this gelatinizing cannot be observed in preserved material, this point was of no value in determining the species in collections studied.

A wide range, both in width of filament and size of oögone, was a striking feature noted in the study of seven different collections of *V. terrestris*. The dimensions given above are not found within any one collection, but are a combination of measurements from these. There was no such great variation in size in material gathered from a single station. For example, in material from one source, the filaments ranged from 38-55 μ in width, and from another from 100-190 μ . The combined minimal and maximal dimensions of filaments of *V. terrestris* as found in California exceed those given by either Collins (1909) or Heering (1921). Similarly, the dimensions of sex organs show greater variation than they report. The series of measurements indicates clearly the slight value of dimensional figures in species determination within this genus.

VAUCHERIA HAMATA (Vaucher) De Candolle

Plate XXVI, figures 12 and 13

De Candolle, 1805, p. 63; Walz, 1866, p. 148, Pl. 12, figs. 12-16, Pl. 13, fig. 17; Wolle, 1887, p. 152, Pl. 127, figs. 12-17; Goetz, 1897, p. 119, figs. 31-34; Heering, 1907, p. 159, fig. 84; 1921, p. 90, fig. 81; Collins, 1909, p. 426; Brown, 1929, p. 98, Pl. 19, fig. 37.

Antherid more or less hooked, terminating the lateral fruiting branch. Oögones one or two, on long, upwardly curved pedicels. Oögones and antherid bent in the same direction but at a distinct angle with each other. Zygote with a single central brown spot and a three-layered wall, the central layer thick and shining.

Width of filaments 37–70 μ . Oögones 55–100 μ long, 50–95 μ broad.

Previous records from California, Illinois, and Washington.

V. hamata is a far less common species than *V. geminata* or *V. terrestris*, which it superficially resembles. Only four of the collections contained material which could be certainly determined as this species. Three of these came from damp, shaded soil on the Stanford campus, and a fourth from a slow-moving stream near Atascadero.

This species is one rather difficult to determine, since at first glance a fruiting branch bearing two oögones resembles *V. geminata*, and one bearing a solitary oögone, when viewed laterally, is easily confused with individuals of *V. terrestris*. *V. hamata* may be distinguished from other Corniculate-Racemose Vaucherias by the long upwardly curved pedicels, and the manner in which the antherid lies in respect to the oögones. In *V. terrestris* the oögone is bent in the same plane in which the antherid is bent. *V. hamata*, on the other hand, has the oögone bent in the same direction as the antherid, but the two are at an angle with one another.

The fact that the oögone wall adheres to the zygote when it falls, and does not gelatinize, as in the case of *V. terrestris*, is difficult to determine, and of little value in identification of preserved material. Asexual reproduction is unknown in *V. terrestris*, while *V. hamata* produces aplanospores.

In spite of its superficial resemblance to the species *geminata* and *terrestris*, *V. hamata* (Vaucher) De Candolle is one worthy of specific rank.

SUB-SECTION RADIATAE

VAUCHERIA GARDNERI Collins

Plate XXVI, figure 15

Collins, 1907, p. 201, Pl. 76, figs. 2–3; 1909, p. 428, Pl. 17, fig. 152; Brown, 1929, p. 100, Pl. 18, fig. 27.

V. geminata forma *pedunculata* Heering, 1907, p. 158, fig. 81.

Antherids and oögones pedicellate, radiating from a restricted, slightly elevated region on the filament. Antheridial stalk long, terminating in a more or less hooked antherid, perpendicular to the filament or nearly so, usually solitary, more rarely two to three. Oögones one to four, ellipsoidal or plano-convex to concave, the flattened side toward the antherid; distinctly beaked, on slender radiating pedicels lying at an acute angle to the filament.

Width of filaments 50–70 μ . Oögones 70–95 μ long, 60–75 μ broad.

Reported from California, Illinois, and Iowa.

This is apparently a rare species, having been found at one station

only, a stream near Los Banos. It is probably aquatic in habitat, since the locality at which it was found and those reported by Collins (1907) are of this type. Professor D. H. Campbell states that the species occurs locally in unusually wet winter seasons when there is an abundance of standing water.

The absence of a distinct fruiting branch distinguishes this species from those of the Racemose type, and the long pedicellate oogones differentiate it from members of the Sub-section Sessilis. The presence of more than one antherid accompanying a group of oogones is a characteristic which makes the species entirely different from others of the Corniculate section. Heering (1907), on the basis of Collins's description, places the alga among forms of *V. geminata*, a treatment which shows that he did not understand the essential point of the species. Tiffany (1926) also seems to have overlooked this extremely striking characteristic when he states (p. 77) that "the presence of distinctly radiating pedicels is about the only characteristic that seems to warrant *V. Gardneri* Collins."

VAUCHERIA GARDNERI var. TENUIS (Collins)

Plate XXVI, figure 16

V. Gardneri forma *tenuis* Collins, 1907, p. 201; 1909, p. 428; Brown, 1929, p. 100.

Oogones and antherids like the type, arising from a restricted area on the filament not perceptibly elevated. Antherids frequently two, more rarely three to four; oogones one to five, rarely more.

Width of filaments 45–65 μ . Oogones 55–80 μ long, 50–70 μ broad.

Reported from California.

Two collections containing material corresponding to the description of the Collins form *tenuis* were available for study. One, it is of interest to note, was made by Professor D. H. Campbell from a pond on the Stanford campus ten years before the species was described by Collins from California material sent him by Gardner. The other was found in a pool near Felt Lake on the Stanford property.

The frequent presence of two or more antherids in a group of fruiting organs, the slightly narrower filaments, and the lack of any elevation of the area on the filament from which the reproductive structures radiate, make this easily recognizable. Collins describes it as a form only, but it seems from this study to be worthy of varietal rank.

SECTION ANOMALAE

VAUCHERIA DEBARYANA Woronin

Plate XXVII, figures 17 and 18

Woronin, 1880, p. 425, Pl. 7, figs. 1–13; Goetz, 1897, p. 130, figs. 51–55; Heering, 1907, p. 167, fig. 91; 1921, p. 95, fig. 85; Brown, 1929, p. 99, Pl. 19, fig. 36.

Antherid terminating the lateral fruiting branch, erect or but slightly bent, broader than the stalk, opening by one to four lateral pores. Oögones one or two, rarely three, spherical to ovoid, with a short terminal beak; borne on erect pedicels below the antherid.

Filaments 20–50 μ broad. Oögones 50–70 μ long, 35–50 μ broad.

This species has not been previously reported from the United States.

This species is apparently rare, having been collected from but two stations. It was found on damp, sandy ground on the bank of a stream at Tres Pinos, San Benito county, and in a similar habitat on the bank of the Pajaro river in Monterey county. In each case it occurred with another species, the filaments of which were considerably larger.

V. Debaryana is the smallest of any of the species studied, and for this reason is easily overlooked in collections. However, it is so distinctive as to be unmistakable, even when the reproductive organs are very immature. It is the only species with lateral fruiting branches in which the stalk bearing the antherid is not distinctly hooked. The erectly borne oögones of ovoid or spherical shape are very different from those of the Corniculate-Racemose group, which are usually flattened or even concave on the side next to the antherid.

According to the original description of Woronin and the reports of later workers, this species, when growing in water, often has calcium carbonate deposited either in the form of occasional crystals or as a continuous sheath on the filaments. However, the amount of acetic acid in the fluid used for preserving the collections is sufficient to dissolve calcium carbonate, and for this reason this point could not be determined.

Although *V. Debaryana* is undoubtedly a rather rare species, the lack of previous records from the United States can be, in part, explained by the unusually small size of its filaments. In collections containing a much larger species, it is quite possible to overlook it entirely. As has been previously noted, it is also possible that algologists have failed to appreciate the value of the structure of the antherid in determining species of *Vaucheria*. Miss Brown's description of this and of *V. Woroniniana* Heering, the other representative of the section *Anomala*, indicates that she, at least, does not understand clearly the nature and significance of this character.

VAUCHERIA WORONINIANA Heering

Plate XXVII, figures 19 and 20

Heering, 1907, p. 165, figs. 89–90; 1921, p. 93, fig. 84; Norrington, 1927, p. 291; Brown, 1929, p. 100, Pl. 18, fig. 29.

V. geminata Goetz, 1897, p. 127, figs. 45–49; Heidinger, 1908, p. 351.

V. Debaryana var. *Schmidlei* Gutwinski, 1903, p. 201.

Fruiting organs borne on lateral branches of varying length. Antherid decidedly broader than the stalk, opening by one or two lateral pores, terminating the fruiting branch, its stalk markedly bent or coiled. Oögones pedicellate, below the antherid, ovoid, with beak slightly inclined toward the antherid.

Width of filaments 50–75 μ . Oögones 70–90 μ long, 65–90 μ broad.

Previously reported from Utah by Miss Norrington.

V. Woroniniana was collected from two stations. It was found growing in great abundance along the edge of a small stream in a shaded ravine in Pacific Grove. It was also collected along the edge of the water at Felt Lake on the Stanford property. In the latter collection the antherids were somewhat immature, but unmistakably of the *Anomalae* type.

In general appearance of the fruiting branch, this species greatly resembles *V. geminata*. The antherid is frequently bent so that its distinctive nature cannot be observed, and considerable study is often necessary in order to determine its type.

Walz (1866) states that certain of his collections of *V. geminata* contained individuals in which the antherid had two lateral pores, instead of the usual single terminal opening. However, he failed to note that this is a constant character worthy of specific rank. Goetz (1897) was the first to recognize the constant nature and value of this point, and applied Vaucher's name *geminata* to this species. As is clearly indicated by the description of Walz (1866), the species *V. geminata* as recognized by him and other early workers is one with the Corniculate type of antherid. For that reason, Heering's application of a new name, *V. Woroniniana*, is the logical treatment for this species.

Since the antherid character is often difficult to determine, especially in immature material, it is quite probable that this species has often been overlooked and confused with *V. geminata*. This is undoubtedly one reason for the single previous record from the United States. Miss Norrington (1927), in reporting *V. Woroniniana* from Utah, fails to place it in her list of algae new to the United States. Her list lacks figures and descriptions, however, and one cannot be certain as to whether or not her material was actually of this species.

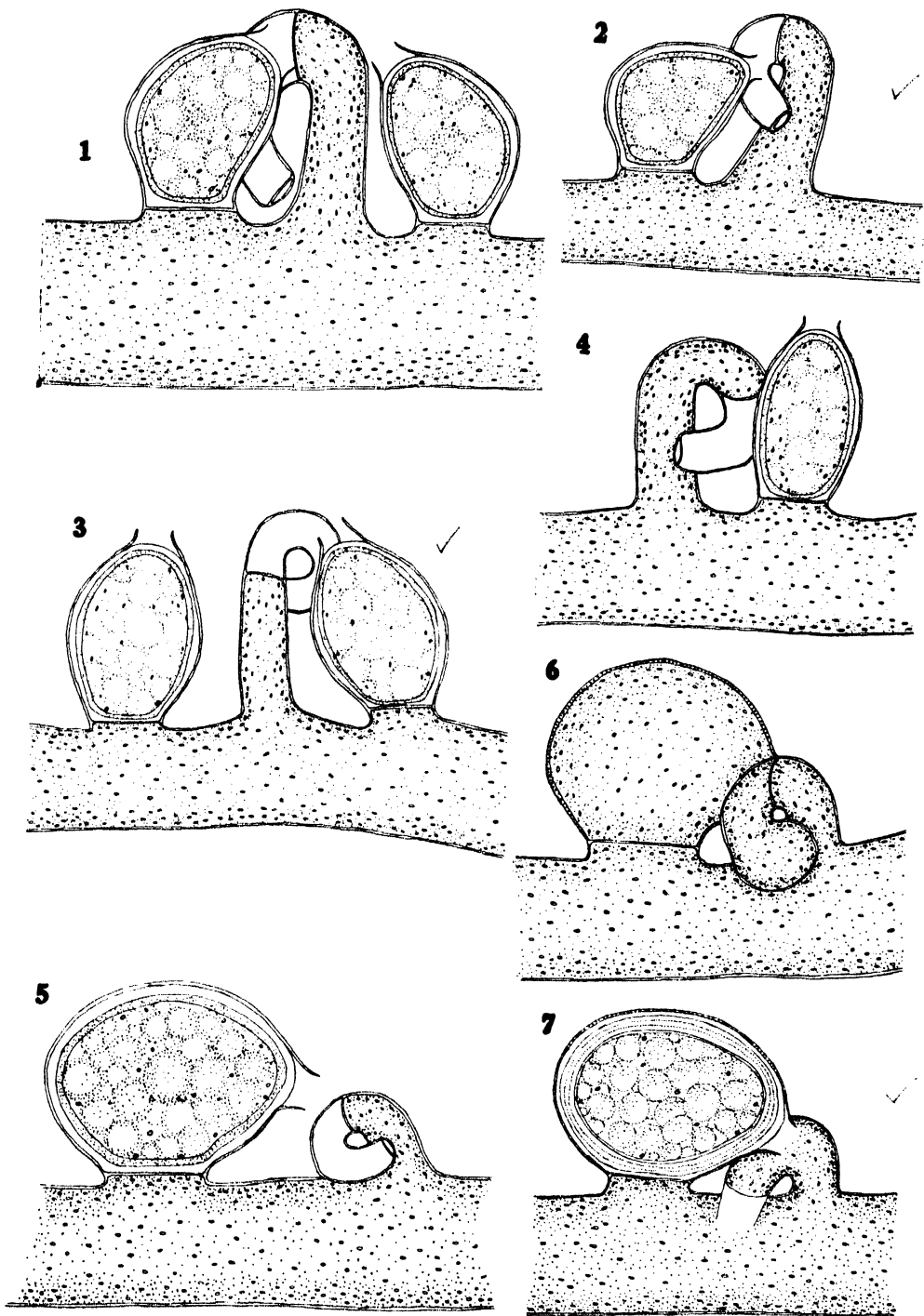
The writer wishes to thank Dr. Gilbert M. Smith for his constant interest and assistance throughout the preparation of this paper.

OCCIDENTAL COLLEGE,
LOS ANGELES, CALIFORNIA

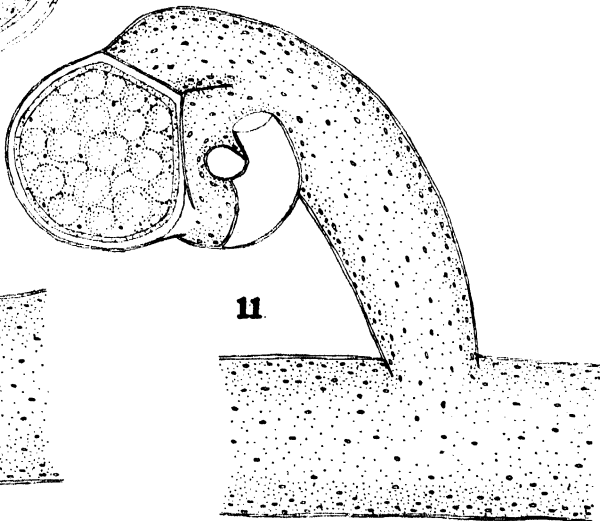
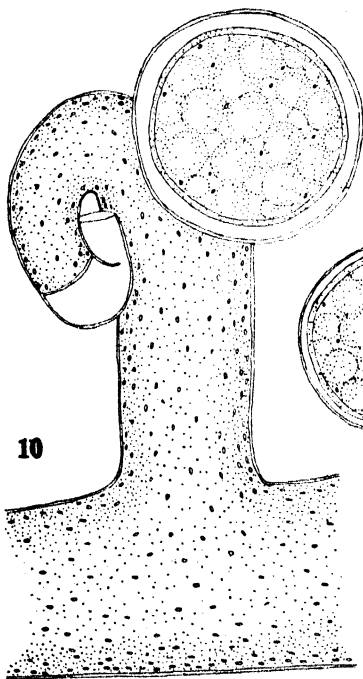
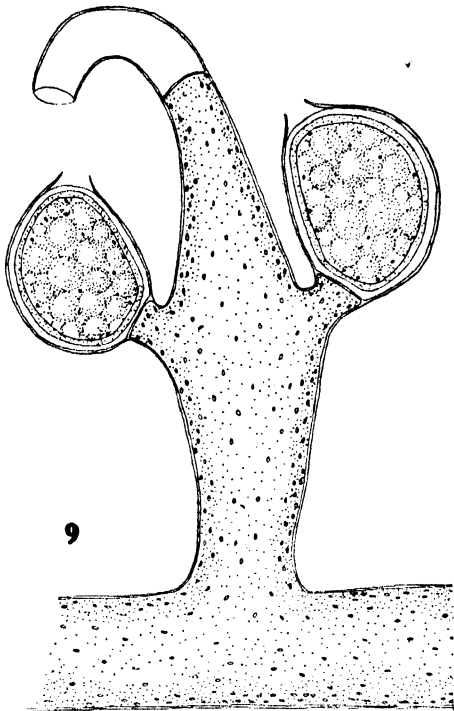
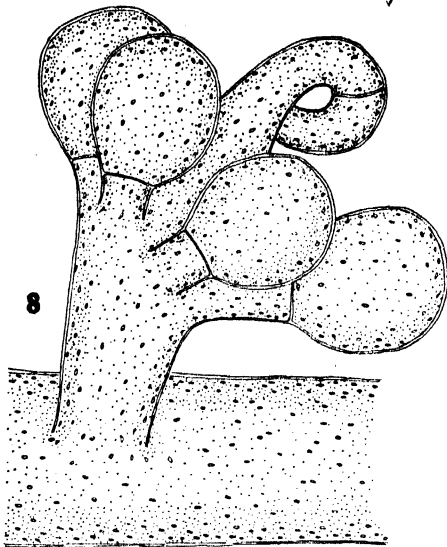
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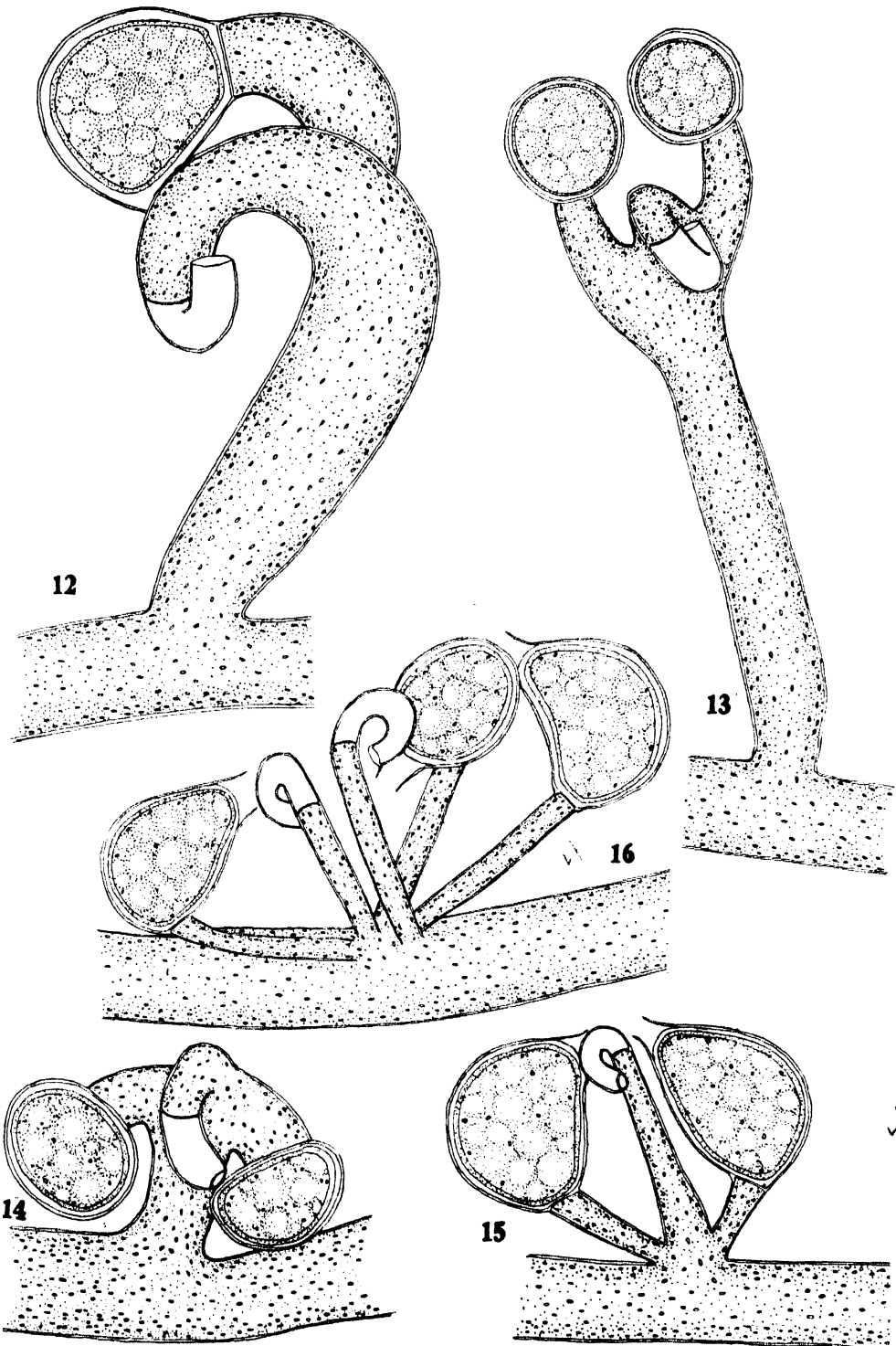
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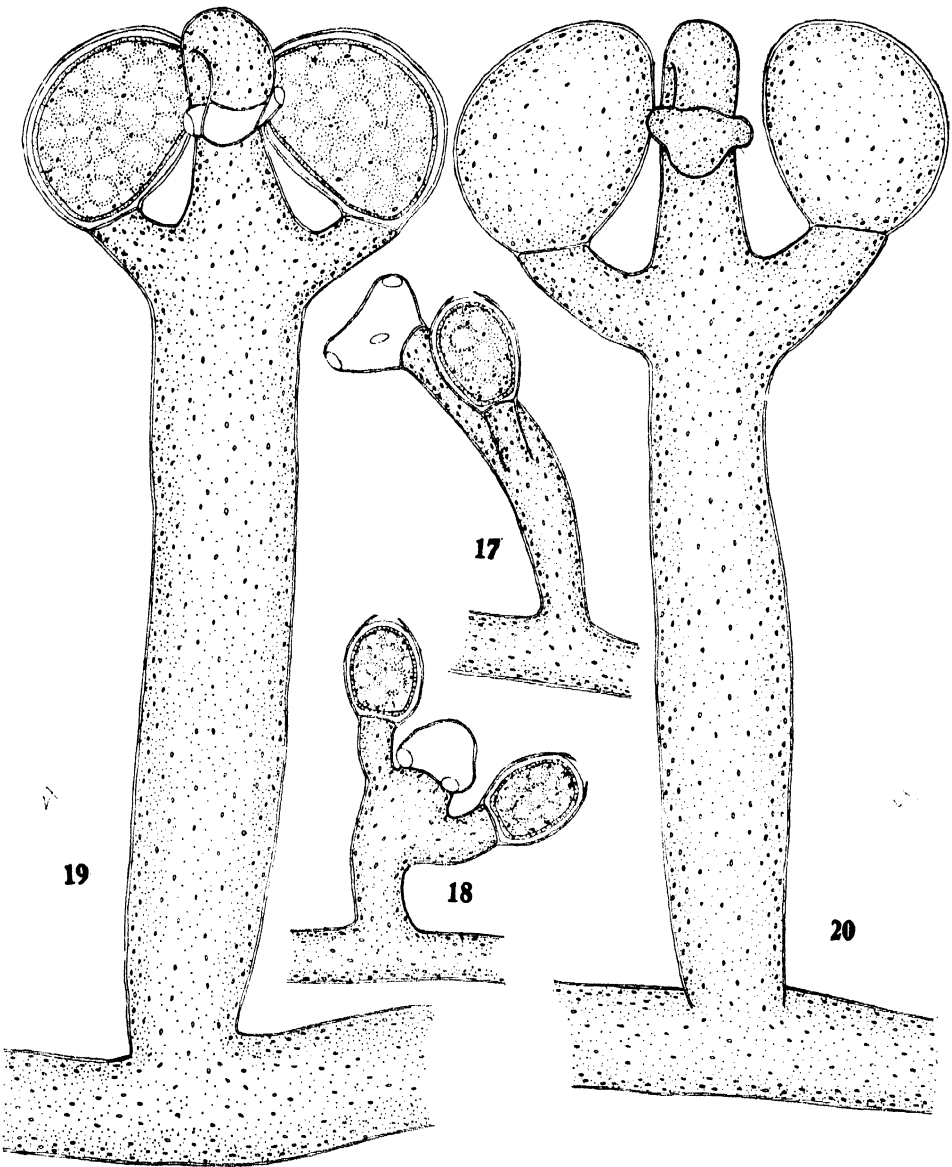
HOPPAUGH: VAUCHERIA



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EXPLANATION OF PLATES

All figures drawn to the same scale with the aid of a camera lucida. Magnification $\times 350$.

PLATE XXIV

FIG. 1. *V. sessilis*.

FIG. 2. *V. sessilis* forma *repens*. ✓

FIG. 3. *V. sessilis* forma *clavata*. Oögone to left of antherid showing intergrading in position of beak to that of *V. sessilis*.

FIG. 4. *V. sessilis* forma *clavata*.

FIG. 5. *V. borealis*.

FIG. 6. *V. pachyderma*. Immature antherid and oögone.

FIG. 7. *V. pachyderma*. Empty antherid and mature zygote.

PLATE XXV

FIG. 8. *V. geminata*. Fruiting branch bearing immature antherid and four immature oögones.

FIG. 9. *V. geminata*. Fruiting branch bearing empty antherid and two mature zygotes.

FIG. 10. *V. terrestris*. Fruiting branch with antherid and oögone bent forward.

FIG. 11. *V. terrestris*. Fruiting branch turned to give lateral view of antherid and oögone.

PLATE XXVI

FIG. 12. *V. hamata*. Lateral fruiting branch bearing empty antherid and single mature zygote.

FIG. 13. *V. hamata*. Lateral fruiting branch bearing empty antherid and two mature zygotes.

FIG. 14. *V. uncinata*.

FIG. 15. *V. Gardneri*.

FIG. 16. *V. Gardneri* var. *tenuis*.

PLATE XXVII

FIG. 17. *V. Debaryana*. Lateral fruiting branch bearing empty antherid with three germ pores and a single mature zygote.

FIG. 18. *V. Debaryana*. Lateral fruiting branch bearing empty antherid with two germ pores and two mature zygotes.

FIG. 19. *V. Woroniniana*. Lateral fruiting branch bearing empty antherid showing two lateral germ pores and two mature zygotes.

FIG. 20. *V. Woroniniana*. Lateral fruiting branch bearing immature antherid and two immature oögones.

THE TOTAL NITROGEN AND CARBOHYDRATES, AND THE RELATIVE RATES OF RESPIRATION, IN VIRUS-INFECTED PLANTS

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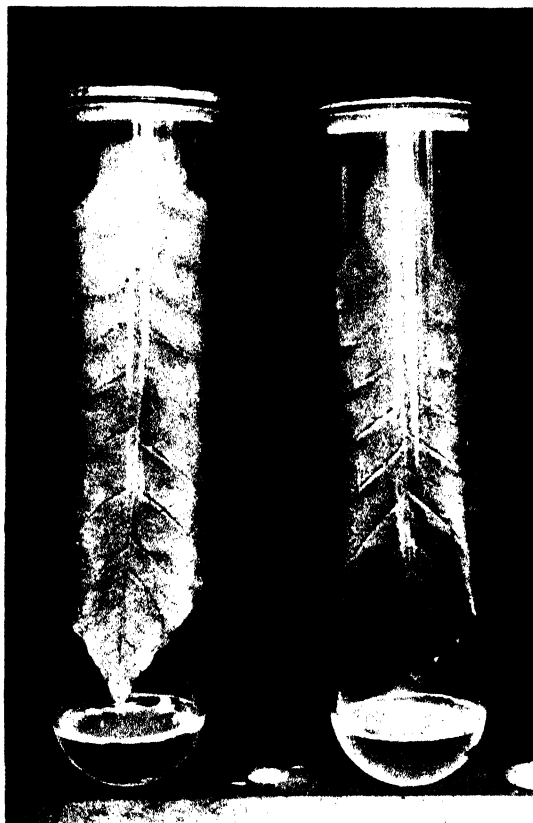
The virus diseases of plants have been most frequently studied with the purpose of determining the nature of the infectious principle of these diseases. The morphological changes produced in host tissues by virus diseases have also been described on many occasions. In a comparatively few instances, investigators have studied the effects of a virus disease upon certain chemical constituents in the host plant. Notable among these latter studies are the investigations of Jodidi (1920) upon the mosaic disease of cabbage, and the works of Jodidi and others (1918, 1920) in connection with certain diseases of the spinach plant. Bunzel (1913) made analyses of the sugar beet affected with the curly-top disease. Brewer, Kendrick, and Gardner (1926) have furnished data upon the effect of mosaic on the nitrogen and carbohydrates of the tomato plant. Rosa (1927) studied the changes in the chemical constituents of the tomato plant brought about by the western yellow blight. Variations in the production of carbohydrates between healthy and blighted spinach plants have been reported by True and Hawkins (1918). Campbell (1925) studied the effects of potato leaf-roll disease upon the carbohydrate, water, and nitrogen content of the host. Microchemical studies of the effects of mosaic disease are reported by Freiberg (1917), in which the contents of diseased cells were compared with those of healthy cells.

The purpose of this investigation was to determine the effects of several virus diseases upon the total nitrogen, upon the total carbohydrates, and upon the respiratory process in the leaves of the respective host plants. First, leaves from plants affected with a certain virus disease were compared with similar leaves from comparable healthy plants of the same species. Secondly, the effects of each virus disease were compared with those of the other diseases studied. An attempt was also made to determine any differences in the effects of the diseases at different ages of the host plants.

MATERIALS AND METHODS

Among the diseases studied were the tobacco, tomato, pokeweed (*Phytolacca decandra*), cucumber, and raspberry mosaics, together with the yellows diseases of peach, plum, aster, and ragweed (*Ambrosia artemisiifolia*). Determinations were also made of the leaf-curl disease of the raspberry. Most of the materials were collected from the field in Connecticut during

the summer of 1928. Certain materials were gathered from the experimental farm and greenhouses of the Connecticut Agricultural Experiment Station. Healthy and diseased leaves were collected at the same time from plants of similar ages which were growing side by side. Care was taken to select healthy leaves of the same age as the diseased leaves, and also to select them from corresponding positions on comparable plants. In regard to the diseased materials, leaves were taken from plants which showed unmistakable symptoms of a particular virus infection and which were



TEXT FIG. 1. Test tubes used in determining the CO_2 production of excised leaves.

also in an actively growing condition. With the exception of the peach and plum trees, which were artificially inoculated, most of the diseased materials came from plants which had become naturally infected under field conditions. From 10 to 40 samples consisting of both healthy and diseased material were collected from various localities on different occasions, in securing data upon each disease studied.

For the nitrogen and carbohydrate analyses, the fresh leaves were dried at 95°C . and then ground to a fine powder. The total nitrogen

determinations were made by the Kjeldahl method modified to include the nitrate nitrogen. The total carbohydrate determinations were intended to include the reducing sugars and most of the acid-hydrolyzable carbohydrates with the exception of the true cellulose. In the carbohydrate analyses, hydrolysis of the leaf powder was carried on for 2 hours with 2 percent HCl at a temperature of 100°. The insoluble leaf residue was then filtered off. The hydrolyzed solution was neutralized, cleared with neutral lead acetate, and delead with potassium oxalate. The reducing power of the cleared filtrate was then determined with Fehling's solution and the residual copper determined by a modification of the iodometric method of Shaffer and Hartman (1921).

In the study of the CO₂ production of healthy and diseased tissues detached leaves were suspended in tightly stoppered test tubes containing definite amounts of barium hydroxid solution. The test tubes were placed in a dark room at a constant temperature of 25° for a period of 10 hours or more. The tubes were shaken frequently to break up the film of carbonate which formed on the surface of the alkali and to prevent accumulation of CO₂ around the leaves. The amount of CO₂ given off by the enclosed leaf was determined finally by titration of the barium hydroxid in the test tube. Blank tubes containing only the hydroxid solution were prepared at the same time and the contents of these tubes were titrated to determine the amount of atmospheric CO₂ which was absorbed.

ANALYTICAL RESULTS

Comparisons were made of the total nitrogen in healthy and diseased leaves of 11 different species of plants. In table I are given the average percentages of total nitrogen found in the dried leaf materials. The percentage gain or loss of nitrogen in the diseased as compared with normal tissues is shown in the last column of table I. Each figure of this column

TABLE I. *Total Nitrogen of Healthy and Diseased Leaves*

| Host | Disease | Nitrogen as Percentage of Dry Weight | | Ratio Diseased : Healthy Percent |
|---------------------------|-----------|--------------------------------------|----------|--|
| | | Healthy | Diseased | |
| Tobacco..... | Mosaic | 5.43 | 5.93 | 110 |
| Tomato..... | Mosaic | 4.33 | 4.70 | 109 |
| Squash..... | Mosaic | 5.39 | 6.18 | 115 |
| Cucumber..... | Mosaic | 4.85 | 5.18 | 107 |
| Pokeweed..... | Mosaic | 2.42 | 2.77 | 114 |
| Pepper..... | Mosaic | 3.15 | 3.25 | 103 |
| Raspberry (1-yr. canes *) | Mosaic | 2.51 | 2.75 | 110 |
| Raspberry (1-yr. canes *) | Leaf Curl | 1.91 | 2.36 | 123 |
| Raspberry (2-yr. canes †) | Mosaic | 1.93 | 1.87 | 97 |
| Peach..... | Yellows | 3.62 | 1.89 | 52 |
| Aster..... | Yellows | 2.72 | 1.37 | 50 |
| Ragweed..... | Yellows | 2.01 | 1.45 | 45 |
| Plum..... | Yellows | 2.48 | 1.73 | 69 |

* Growth of present year.

† Growth of previous year.

is obtained by dividing the number of milligrams of nitrogen found in one gram of diseased material by the corresponding content in the same amount of healthy material.

The mosaic diseases, with the single exception of the mosaic leaves from the two-year raspberry canes, were found to be accompanied by an increase in the nitrogen content of the diseased leaves, on a percentage of dry weight basis. This increase varies from 3 percent of the normal content in the case of the mosaic pepper leaves to 23 percent in the case of the leaf-curl disease of the raspberry. The 4 species of plants affected with yellows diseases show a marked decrease, varying from 17 to 50 percent of the normal amount, in the nitrogen content of the diseased material. The decreases in total nitrogen caused by the yellows diseases were much greater than the increases of this constituent in the diseased leaves of the mosaic plants.

The total carbohydrates were determined in samples of the same materials which were used in the previous total nitrogen analyses. The results of these determinations are given in table 2. In this table the

TABLE 2. *Total Carbohydrates of Healthy and Diseased Leaves, as Dextrose*

| Host | Disease | Carbohydrates as Percent- age of Dry Weight | | Ratio Diseased : Healthy Percent |
|----------------------------|-----------|--|----------|--|
| | | Healthy | Diseased | |
| Tobacco..... | Mosaic | 16.8 | 12.9 | 77 |
| Tomato..... | Mosaic | 19.4 | 15.8 | 81 |
| Squash..... | Mosaic | 13.4 | 11.5 | 86 |
| Cucumber..... | Mosaic | 18.1 | 13.6 | 75 |
| Pokeweed..... | Mosaic | 22.7 | 17.8 | 78 |
| Pepper..... | Mosaic | 10.7 | 10.0 | 93 |
| Raspberry (1-yr. canes)... | Mosaic | 23.4 | 22.6 | 96 |
| Raspberry (1-yr. canes)... | Leaf Curl | 20.1 | 19.2 | 95 |
| Raspberry (2-yr. canes)... | Mosaic | 18.1 | 19.2 | 106 |
| Peach..... | Yellows | 20.8 | 39.6 | 190 |
| Aster..... | Yellows | 27.2 | 53.6 | 197 |
| Ragweed..... | Yellows | 7.5 | 12.8 | 171 |
| Plum..... | Yellows | 27.5 | 37.3 | 136 |

amounts of carbohydrate materials, determined as dextrose, are expressed as percentages of dry weight. The mosaic diseases, with one exception, were found to be accompanied by a decrease of the total carbohydrates in the leaf tissues. The mosaic leaves from two-year raspberry canes and leaves of all the plants affected with yellows diseases were found to have a higher carbohydrate content than corresponding leaves of healthy plants. In the case of the mosaic diseases, the increase of total carbohydrates was found to vary from 5 to 27 percent among the different host species, while the decrease of carbohydrates in the case of the yellows diseases was found to vary from 6 to 97 percent of the amounts in healthy tissues. As in the case of total nitrogen, the total carbohydrates were found to undergo greater changes in amounts in the yellows than in the mosaic diseases.

The total nitrogen and total carbohydrate contents determined in the above analyses were also calculated upon an absolute basis, as the number of milligrams of a constituent in a single average leaf. Results expressed on this single leaf basis were generally similar to those on the percentage basis. Since more diseased than healthy leaves were usually required to furnish a given amount of dried material, certain consistent differences were noted in the expression of the data on the two bases. An increase in amount of a leaf constituent due to the diseased condition, as expressed on a percentage basis, became less significant or occasionally appeared as a decrease when expressed on the absolute basis.

Carbohydrate/nitrogen ratios were calculated from the percentage amounts of total carbohydrates and total nitrogen found in the leaf materials, in the case of both healthy and diseased plants. As may be seen in table 3, these ratios in the case of mosaic plants are less than those

TABLE 3. *Carbohydrate/Nitrogen Ratios of Healthy and Diseased Leaves*

| Host | Disease | C/N Ratio | | Ratio Diseased : Healthy Percent |
|----------------------------|-----------|-----------|----------|--|
| | | Healthy | Diseased | |
| Tobacco..... | Mosaic | 3.1 | 2.2 | 71 |
| Tomato..... | Mosaic | 4.5 | 3.4 | 76 |
| Squash..... | Mosaic | 2.5 | 1.9 | 76 |
| Cucumber..... | Mosaic | 3.7 | 2.6 | 70 |
| Pokeweed..... | Mosaic | 9.5 | 6.4 | 67 |
| Pepper..... | Mosaic | 3.4 | 3.0 | 88 |
| Raspberry (1-yr. canes)... | Mosaic | 9.4 | 8.1 | 86 |
| Raspberry (1-yr. canes)... | Leaf Curl | 10.6 | 8.0 | 75 |
| Raspberry (2-yr. canes)... | Mosaic | 9.5 | 10.1 | 106 |
| Peach..... | Yellows | 5.8 | 21.0 | 362 |
| Aster..... | Yellows | 10.1 | 38.3 | 379 |
| Ragweed..... | Yellows | 3.8 | 8.5 | 224 |
| Plum..... | Yellows | 11.0 | 21.9 | 199 |

for corresponding healthy materials. The yellows diseases show an increased carbohydrate/nitrogen ratio in the diseased leaves. In the last column of table 3 are given the percentage decrease or increase of the ratios of these two leaf constituents in diseased material as related to healthy material. The mosaic diseases were found to cause decreases in the carbohydrate/nitrogen ratio varying from 12 to 33 percent and the yellows diseases were found to be accompanied by increases in this ratio varying from 6 to 279 percent of similar ratios for normal plants.

Studies were made of the amounts of CO_2 produced in the dark by detached leaves of 7 species of plants, in both healthy and diseased conditions. The rates of respiration were determined in milligrams of CO_2 produced in 1 hour by an average leaf. The rates for both healthy and diseased leaves are given in table 4. Each rate given in this table represents the average of from 15 to 40 experiments. It was found that 6 of the 9 diseased conditions investigated were accompanied by decreases in the

amounts of CO₂ produced by the excised leaves as compared with the CO₂ production of detached healthy leaves. The increase in CO₂ production in the case of the mosaic and leaf-curl diseases of the one-year raspberry canes may be attributed to the fact that the leaves came from stems which were comparatively young. Considering only the mature, diseased leaves from fully developed plants, the aster was the only species studied which produced more CO₂ than was produced by comparable healthy leaves of the same species.

Rates of CO₂ production based upon the fresh weight of the leaves showed variations similar to those given in table 4 on the single leaf basis, between healthy and virus-infected tissues.

TABLE 4. *Rates of Respiration of Healthy and Diseased Leaves*

| Host | Disease | CO ₂ per Leaf per Hour (mg.) | | Ratio Diseased : Healthy Percent |
|----------------------------|-----------|---|----------|--|
| | | Healthy | Diseased | |
| Tobacco..... | Mosaic | 1.318 | 1.022 | 77 |
| Tomato..... | Mosaic | .170 | .163 | 96 |
| Pokeweed..... | Mosaic | .178 | .150 | 84 |
| Cucumber..... | Mosaic | .235 | .173 | 74 |
| Raspberry (1-yr. canes)... | Mosaic | .074 | .093 | 125 |
| Raspberry (1-yr. canes)... | Leaf Curl | .057 | .068 | 119 |
| Raspberry (2-yr. canes)... | Mosaic | .144 | .114 | 79 |
| Peach..... | Yellows | .210 | .150 | 71 |
| Aster..... | Yellows | .225 | .284 | 126 |

Effects of the mosaic disease upon the total nitrogen, total carbohydrates, and respiration in young tobacco leaves were compared with the effects upon older tissues. The leaves of young plants and the youngest leaves of older plants were found to be of similar composition and to show the same variations in the diseased condition. The largest actively-functioning leaves of nearly full grown tobacco plants were used in securing the data upon mature tissues. The results of these studies, given in table 5, show

TABLE 5. *Effects of Mosaic Upon the Nitrogen, Carbohydrates, and Respiration in Young and Mature Leaves of Tobacco*

| | Young Tissues | | | Mature Tissues | | |
|---|---------------|--------|------|----------------|--------|------|
| | Healthy | Mosaic | M/H | Healthy | Mosaic | M/H |
| Nitrogen (percent dry wt.)..... | 5.78 | 6.33 | 110% | 5.11 | 5.49 | 107% |
| Carbohydrates (percent dry wt.)... | 15.9 | 11.6 | 73 | 17.2 | 13.3 | 78 |
| CO ₂ (mg. per leaf per hr.)..... | .173 | .193 | 112 | 1.48 | 1.13 | 76 |

the increase of nitrogen in the young, diseased leaves of tobacco to be somewhat greater than the increase in similar mature leaves. Also, the young diseased leaves were found to undergo the greater reduction in

amount of carbohydrate materials. CO_2 production was found to be increased by the diseased condition in the young tissues, whereas in the mature tissues there was a large decrease in the respiratory rate, as compared with normal leaves. All these determinations seem to indicate that young tobacco tissues are affected to a greater degree than mature tissues, by the mosaic disease.

Growth measurements of healthy and mosaic tobacco plants were made throughout the growing period. It was found that the average height of the mosaic plants was less at all periods of growth than that of healthy plants. The most rapid increase in height of both healthy and diseased plants occurred during a period just previous to blossoming. This period of rapid growth was found to begin in the mosaic plants about 15 days earlier than in the healthy plants. The healthy plants continued to grow for about 2 weeks after the mosaic plants had reached their maximum height and finally averaged 20 cm. taller than the mosaic plants.

In all the virus diseases studied, a decrease was noted in the fresh and dry weights of the diseased leaves as compared with similar healthy leaves. No consistent variations were found in the percentage moisture or dry matter, between the normal and diseased materials.

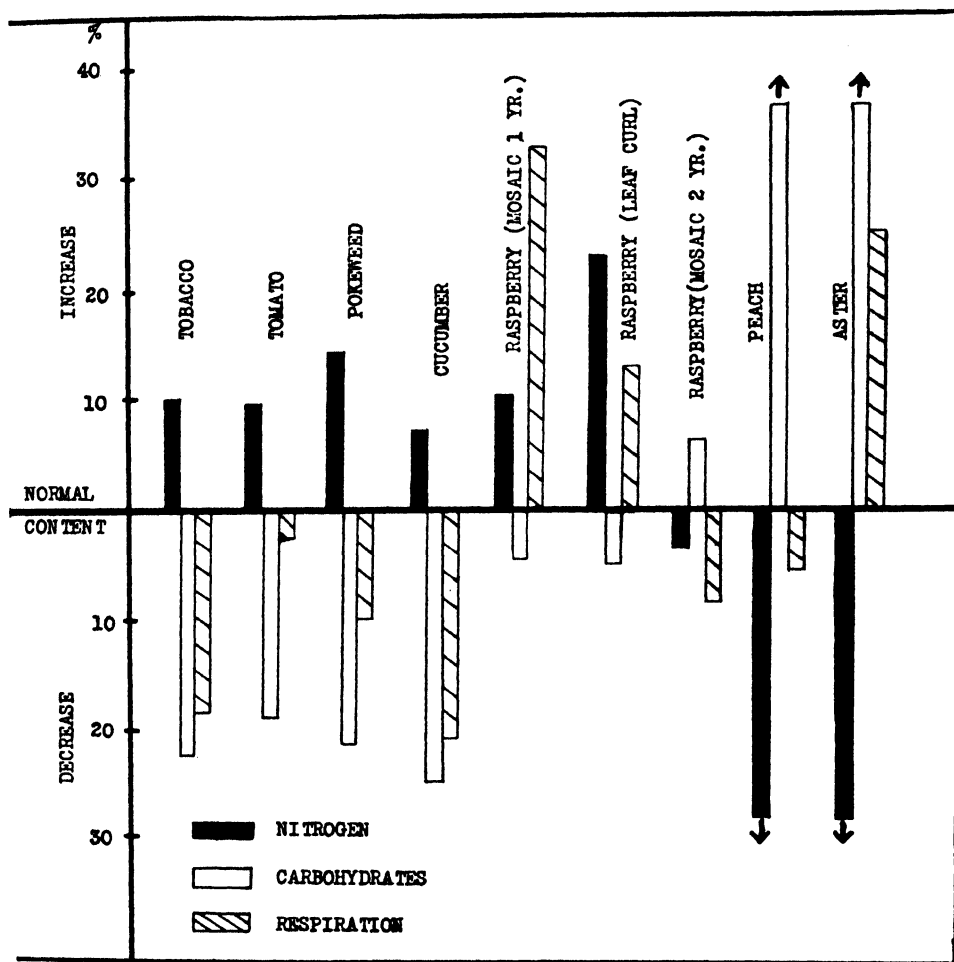
DISCUSSION

The results of the total nitrogen and total carbohydrate analyses made in this investigation seem to indicate that the diseases studied may be divided into two classes, according to the effects of the disease upon these two leaf constituents. The first of these classes consists of diseases which cause an increase in the total nitrogen and a decrease in the total carbohydrates of the host. The diseases in this class are all commonly designated as mosaics. The second group consists of those diseases which were found to be accompanied by lower nitrogen and higher carbohydrate contents in the diseased than in normal tissues. Most of the diseases in this class are of the yellows type. Cook (1926) has suggested that such a grouping of diseases might exist, upon finding that the photosynthetic activities of mosaic sugar cane were greatly reduced, while in the case of the peach yellows and little peach diseases there was found an accumulation of starch; these studies, however did not involve the total carbohydrate content of the diseased tissues. No statement has been found in the literature to the effect that increased carbohydrate contents in plants affected with virus diseases are accompanied by decreased nitrogen contents or that a high nitrogen content is accompanied by a reduction of carbohydrates as has been found in this investigation.

Text figure 2 shows diagrammatically that the diseases studied produced opposite effects upon the total nitrogen as compared with the total carbohydrate content of the host plants. It may be noticed in this figure that the leaf-curl disease of the raspberry would be placed among the mosaic

diseases. The mosaic leaves of the two-year raspberry canes show the characteristic effects of the yellows diseases, whereas the diseased leaves from one-year canes resemble the mosaic diseases in their variations in leaf constituents. Such a condition may be due to the prolonged effect of the mosaic virus upon the perennial parts of the host plant.

In regard to the differences in the effects of virus diseases upon the



TEXT FIG. 2. Diagram showing variations in nitrogen, carbohydrates, and respiration, accompanying the diseased conditions.

nitrogen content as compared with the effects upon the carbohydrate content of the host plants the results of a few investigators who have worked with certain specific diseases may be mentioned. Comparative analyses made by Bailey (1924) of healthy and "calicoed" leaves of tobacco, tomato, and petunia showed an increased nitrogen content and a decrease of starch and soluble carbohydrates in the diseased materials. Upon comparing light and dark areas of leaves affected with mosaic disease Freiberg

(1917) found that carbohydrates were more abundant in the dark green than in the light green areas, and a slight excess of protein was found in the lighter areas. Leaves of curly-top sugar beets according to analyses by Bunzel (1913) contained less sugar than healthy leaves while the nitrogen content remained unchanged. In the roots of these plants however, an increase of total nitrogen was found together with a decrease in the sugar content. Effects similar to those found in the case of the yellows diseases investigated in this work, have also been reported by others. Rosa (1927), in work upon the western yellow blight of tomato, found a decrease of total nitrogen in the diseased leaves and an accumulation of carbohydrates in all parts of the blighted plants. In connection with the blight disease of spinach, True and Hawkins (1918) found an accumulation of carbohydrates in the blighted leaves; while Jodidi and others (1920) report a decrease of total nitrogen in the diseased material. Campbell (1925) has found the leaf roll disease of potato to be accompanied by a higher percentage of carbohydrates, while the nitrogen percentage of the dry weight in disease was approximately the same as that of healthy plants. It seems logical, therefore, that many of the virus diseases could be classed either as mosaic or as yellows diseases according to the changes induced in the nitrogen and carbohydrate contents of the host tissues.

Studies of the respiratory rates of detached leaves of healthy and diseased plants have in most cases shown that mature, diseased leaves produce less CO_2 than similar healthy leaves, in the case of both the mosaic and yellows types of diseases. If the rate of production of CO_2 may be considered as an index of protoplasmic activity, it might be inferred that the diseased tissues were less active than the healthy tissues. Since the respiratory rates of young tobacco leaves were found to be somewhat increased by the diseased condition, the decrease in this rate which was found in the older tissues may be due to protoplasmic fatigue brought about by over-stimulation of these tissues through the presence of the virus during an earlier period in the development of the leaves. There seems to be no consistent evidence that variations in the respiratory rate due to the diseased condition are brought about by variations in the nitrogen or carbohydrate contents of the leaves examined.

SUMMARY

1. The virus diseases studied in this investigation may be divided into two classes, mosaic diseases, and yellows diseases, according to the effects of the disease upon the total nitrogen and total carbohydrate contents of the leaves of the host plants.

2. Mosaic diseases were found to be accompanied by an increase in the total nitrogen and by a decrease in the total carbohydrate contents of the diseased leaves.

3. The yellows diseases were accompanied by a reduction in total

nitrogen and by an increase of total carbohydrates, as compared with healthy leaf material.

4. The carbohydrate/nitrogen ratio was decreased in the mosaic leaves and increased in leaves affected with yellows diseases.

5. Yellows diseases caused greater variations in the amounts of constituents in the leaves of their hosts than did mosaic diseases.

6. Respiration was found to be increased in young tissues affected with virus diseases.

7. Respiration was decreased in older diseased leaves as compared with healthy material.

This investigation was carried out at the Osborn Botanical Laboratory, Yale University. Much of the material used was secured through the Connecticut Agricultural Experiment Station. The author wishes to express his appreciation of suggestions and advice received from Dr. C. G. Deuber and Dr. G. P. Clinton.

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STUDIES IN ONAGRACEAE¹ V. THE NORTH AMERICAN
SPECIES OF THE SUBGENERA LAVAUXIA
AND MEGAPTERIUM OF THE
GENUS *OENOTHERA*

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(Received for publication January 10, 1930)

In the preceding papers of this series there have been treated the North American species of the subgenera Chylismia, Sphaerostigma (Chamissonia), Eulobus, Calylophis (Meriolix), Salpingia (Galpinsia), and Taraxia, or the Onagreae-Chamissoniinae of Raimann (Engler & Prantl, Natürl. Pflanzenfam., III, Abt. 7: 216. 1893). This group includes those members of the genus with the stigma spherical or discoid. The present paper begins with those subgenera having the stigma divided into four lobes, taking up the discussion of two of these, namely Lavauxia and Megapterium. Of the former I have had insufficient South American material to include such species; the latter is exclusively North American.

In both of these subgenera, the seeds are winged at the somewhat flattened apex. In Lavauxia, the capsule itself is ovoid, quite woody in texture, with angles winged most widely in the upper half so as to make the capsule appear obpyramidal, and with the seeds merely granular and in two rows in each cell of the capsule. In Megapterium the capsule is more cylindrical or prismatic, less indurate, with the angles usually winged their entire length, and with the seeds having corky tubercles and occurring in one row in each cell of the capsule. With such a basis of division, I would place the North American species as follows: (1) in Lavauxia, *Oenothera triloba*, *O. flava*, *O. taraxacoides*; (2) in Megapterium, *O. missouriensis*, *O. brachycarpa*, *O. Fremontii*, and *O. graminifolia*. Of other species that have been referred to Lavauxia, I would place in Pachylophis: *O. primiveris* (including *Lavauxia lobata* A. Nels.) and *O. tubifera*. Of those that have been referred to Megapterium, I would place in Hartmannia: *O. dissecta* and *O. Havardi*, and in Gaurella: *O. canescens*.

The transfer of *Oenothera brachycarpa* from Lavauxia to Megapterium differs from the usual disposition, but the basis of being acaulescent or caulescent, which has been so often used to separate the two groups, means little. I have seen specimens of the South American species *O. acaulis*, which is an undoubted Lavauxia, with stems several decimeters long. The acaulescent condition of *O. brachycarpa* does not deter me therefore, from placing the species in Megapterium, where various other characters seem to place it; namely, capsule-wings running from base of fruit rather

¹ No. IV of this series appeared in Amer. Jour. Bot. 16: 702-715. 1929.

than apex, seeds in 1 row in each cell of the capsule, and seeds with corky tubercles.

LAVAUXIA

For the study of this group I have had material from the herbaria here-with listed, for which the accompanying abbreviations are used in citing specimens: University of California (C), Gray Herbarium (G), Missouri Botanical Garden (M), Pomona College (P), and Stanford University (S). I hereby express my indebtedness to those who have kindly loaned me the material used.

Subgenus **Lavauxia** (Spach) Jeps., Man. Fl. Pls. Calif., 680. 1925

Lavauxia as genus, Spach, Hist. Veg. Phan. 4: 366. 1835; Raimann, l.c.; Small, Bull. Torrey Club 23: 182. 1896. *Lavauxia*, as section, Endlicher, Gen. Pl., 1190. 1840.

Annual to perennial, acaulescent or nearly so. Leaves more or less pinnatifid. Flowers vespertine, white to yellowish, the calyx-tube much elongated. Stigma with 4 linear lobes. Capsule woody, the body ovoid, with 4 winged angles, at least above. Seeds in 2 rows in each cell, slightly granular, cuneate-obovoid, slightly concavo-convex, with wing-like margin around obtuse summit.

Type species, *Oenothera triloba* Nutt.

KEY TO SPECIES

- Calyx-tube 2-12 cm. long, usually not more than two-thirds the length of the leaves; calyx-lobes 10-18 mm. long; petals 10-20 mm. long; capsules ovoid, with wings 2-10 mm. wide and developed for a considerable portion of the length of the capsule.
- Capsules with broad wings (5-10 mm. wide), and terminal expanded valve-like teeth 1.5-3 mm. long; leaves thin, divided almost to midrib which has a green margin expanding suddenly into ovate or lance-ovate terminal lobe; calyx greenish; petals drying whitish; slender-rooted winter annuals or biennials of low altitudes from Kansas to Tenn. and Texas. 1. *O. triloba*.
- Capsules with narrower wings (2-5 mm. wide) and terminal teeth 0.5-1.5 mm. long; leaves rather thick, usually not so deeply divided and insensibly widened into lance-linear or lanceolate terminal lobe; calyx frequently drying purplish, as do the petals; heavy-rooted perennials of middle to high altitudes from Canada through the Rocky Mts. to Chihuahua and west to Calif. 2. *O. flava*.
- Calyx-tube 15-20 cm. long, often quite or nearly equaling the length of the leaves; calyx-lobes 35-40 mm. long; petals 30-40 mm. long; capsules cylindric-ovoid, with wings at summit only or quite lacking, not over 1-2 mm. wide. Mts. of southern N. Mex., Ariz., into Chihuahua. 3. *O. taraxacoides*

TREATMENT OF SPECIES

1. **OENOTHERA TRILOBA** Nutt., Journ. Acad. Philad. 2: 118. 1821
O. triloba in T. & G., Fl. N. Am. 1: 499. 1840; Wats., Proc. Am. Acad. 8: 586. 1873, in part. *Lavauxia triloba*, by inference, Spach, Hist.

Veg. Phan. 4: 367. 1835; Raimann, in Engler & Prantl, Natürl. Pfl. fam. III, Abt. 7: 215. 1893; Small, Bull. Torr. Club 23: 182. 1896, in part. *Oenothera triloba* var. *parviflora* Wats., Proc. Am. Acad. 12: 251. 1876. *Lavauxia triloba* var. *Watsoni* Britton, Mem. Torr. Club 5: 235. 1894; Small, Bull. Torr. Club 23: 182. 1896. *Lavauxia Watsoni* (Britton) Small, Fl. SE. U. S., 844 and 1335. 1903. *Oenothera rhizocarpa* Sprengel, Syst. 2: 230. 1825. *Lavauxia Nuttalliana* Spach, Nouv. Ann. Mus. Paris 4: 358. 1835. *Oenothera Roemeriana* Scheele, Linnaea 22: 154. 1849. *O. clandestina* Nutt., in Wats., Proc. Amer. Acad. 8: 615. 1873, in synonymy; Small, l.c. *O. taraxacifolia* Leveille & Guffroy, ex Levl., Mon. Onoth., 73. 1902, in part.

Winter annual to biennial (or possibly short-lived perennial), commonly subcaulescent, occasionally with few stems up to 2 dm. long, glabrate throughout or finely strigillose and even glandular, especially on calyces, ovaries, and to a lesser extent on veins and leaf-margins; root generally a slender taproot; leaves usually in close tuft; leaf-blades thin, oblanceolate in outline, 3–20 (30) cm. long, 1–4 (7) cm. wide, deeply and regularly runcinate-pinnatifid almost to midrib, the lateral lobes acute to acuminate, dentate to serrate, divergent and often recurved, lanceolate to linear, numerous, gradually reduced toward the base of the rachis, with secondary smaller lobes often between larger ones, terminal lobe lanceolate to ovate, acute, larger than lateral ones; petioles slightly or not at all winged, shorter than blade; flowers usually much exceeded by the leaves, vespertine; calyx-tube very slender, 2–10 (15) cm. long, gradually expanded above, finely pubescent within the tubular portion; calyx-lobes distinct or united in anthesis, reflexed, lance-linear, green, 10–18 mm. long, 2–4 mm. wide, with free tips an additional 6 mm. long; petals pale yellow, delicate, 10–20 mm. long, 3–5 veined, orbicular-obovate, sometimes with a small tooth-like lobe in the sinus, so as to appear shallowly trilobed; stamens subequal, filaments somewhat flattened, ca. two-thirds the length of the petals; anthers 5–9 mm. long, glabrous; style glabrous, about as long as stamens; stigma-lobes 3–4 mm. long; capsule very hard, ovoid, 10–20 mm. long, 4-winged, each wing reticulate-veined, 5–10 mm. wide, especially above, often with a tooth about or above the middle, this tooth pointing downward, each wing terminating in a spreading valve-like lobe 1.5–3 mm. long; seeds numerous, dark brown, slightly concave and with a carinate ridge on the ventral side, 2 mm. long, with a narrow wing-like margin around the obtuse summit.

Type locality, "In the arid and partly denuded prairies of Red river." Representative material, KANSAS: Wichita, *Poole* 261 (G); Montgomery Co., *A. S. Hitchcock* 1142 (G, M); buffalo wallows, Ellis Co., type of var. *parviflora*, *Dr. L. Watson in 1876* (G). MISSOURI: Noel, *Bush* 5616 (G, M); Desoto, *Hasse in 1887* (S). KENTUCKY: Bowling Green, *Price in 1891* (M). TENNESSEE: Fosterville, Rutherford Co., *Eggert in 1898* (C, M); Nashville, *Gattinger* 913 (G, M). OKLAHOMA: Catoosa, *Bush* 1167 (M); Alva, Woods Co., *Stephens* 657 (G, M, S); Kingfisher, *Diehl in 1899* (P). TEXAS: New Braunfels, *Lindheimer* 812 (C, G, M, P); San Antonio, *Clemens* 699 (M, P);

Dallas, *Tedmann in 1908* (M); Comanche, *Eggert in 1900* (M); Austin, *Young 98* (G, M). Without definite locality: Red River plains, *Nuttall*, labeled *O. clandestina* in Nuttall's writing (G); Horti Tonelle, *Audibert in 1849*, from Bentham, labeled *O. rhizocarpa* (G); Hort. bot. Budapest, in 1909, caulescent (M); Univ. Calif. bot. Garden, *K. Brandegee in 1913*, caulescent (C, G, M). LOWER CALIFORNIA: San Julio, *T. S. Brandegee in 1889* (C); San Jorge, *T. S. Brandegee in 1889* (C).

In some of the literature, such as Small, *Man. S.E. U.S.*, 844, 1903, the color of the flowers is given as white or pink. One cannot be certain from herbarium specimens, but so far as I can ascertain, all the statements made from living plants give the color as yellow or yellowish (Nuttall, in the original description; Engelmann & Gray, *Pl. Lindheimerianae*, 189. 1850; Sims, *Bot. Mag.*, t. 2566. 1825) as do herbarium labels by K. Brandegee, Price, and as does a letter from Professor Tharp of the University of Texas. Professor Tharp writes further that in Texas this species behaves as a winter annual, which statement agrees well with Nuttall's and Engelmann's observations and with the appearance of most herbarium plants. But *Bush 5616* from Noel, Mo. has dead fruits of the preceding season and flowers of the year collected, 1909. Other specimens would indicate that sometimes the species may be at least biennial. Plants from Lower California, although far removed geographically from the usual range of the plant, are quite typical and with the characteristic fruit with wings widest above the middle, toothed at the middle and with terminal valves.

2. *Oenothera flava* (A. Nels.) Munz, n. comb.

Lavauxia flava A. Nels., *Bull. Torrey Club* 31: 243. 1904. *Oenothera triloba* var. *ecristata* M. E. Jones, *Proc. Calif. Acad. ser. 2*, 5: 681. 1895. *O. taraxacifolia* var. *ecristata* Leveille & Guffr., ex Levl., *Mon. Onoth.*, 77. 1902. *Lavauxia palustris* Rose, *Contr. U. S. Nat. Herb.* 12: 294. 1909. *L. hamata* Woot. & Standl., *Con. U. S. Nat. Herb.* 16: 154. 1913.

General habit that of *O. triloba*, but with perennial thickened tap-root often crowned with leaf-bases and fruits of several previous seasons; leaf-blades oblong-linear to oblanceolate in outline, thicker, narrower (1-2 cm. wide), with terminal lobe narrower (lanceolate to lance-linear), lateral lobing less deep so that rachis is usually more broadly winged and passes gradually into the terminal lobe; flowers as in *triloba*, but petals not at all trilobed and drying purplish as does the calyx often; capsule as in *triloba*, but with wings narrower (2-5 mm. wide) and not toothed, and with terminal valves shorter (0.5-1.5 mm. long); seeds as in *triloba*.

Type locality, Laramie, Wyoming. Ranging widely in the Rocky Mts. Representative material, CANADA: Saskatchewan, *Bourgeau in 1858* (G); Assiniboia, Crane Lake, *Macoun in 1894* (G). WASHINGTON: Yakima River, Morgans Ferry, *Suksdorf 307* (G). IDAHO: Tikura, Blaine Co., *Nelson & Macbride 1302a* (G); Blackfoot, *Henderson 4580* (G). MONTANA:

Bozeman, *Blankinship* 204 (M); Deer Lodge Valley, *M. E. Jones in 1905* (P). NORTH DAKOTA: Dickinson, *Holgate in 1908* (G). WYOMING: Laramie, *Nelson* 219, type coll. (G, M); Ft. Bridger, *Porter* 2319 (M). COLORADO: Mancos, *Eastwood in 1892* (C); Arboles, *Baker* 494 (G, M, P); Steamboat Springs, *Osterhout* 2794 (P); Colorado Springs, *Jones* 957 (P); Middle Park, *Patterson in 1875* (G). UTAH: Geyser Reservoir, San Juan Co., *Walker* 250 (G); Gogorza, Summit Co., *Garrett* 2963 (P); Kanab, type of var. *ecristata*, *Jones* 5274 (C, M, P); Marysville, *Jones* 5397m (C, M, P). ARIZONA: Peach Springs, *J. G. Lemmon & wife in 1884* (C, US); Ash Fork, *Rusby* 606 (C, M); Flagstaff, *Jones in 1884* (P). NEVADA: Glendale, *K. Brandegee in 1913* (C, G, M); Genoa, *Jones in 1897* (M, P); Mountain City, *Nelson & Macbride* 2182 (G, P). CALIFORNIA: lava beds, *M. S. Baker in 1893* (C, M, S); Sierra Co., *Lemmon* 86 (G, M); Fall River Springs, Shasta Co., *Hall and Babcock* 4198 (C). NEW MEXICO: Silver City, *Eastwood* 8388 (G); Socorro, *Vasey in 1881*, type of *hamata* (US); Pecos River, *Coghill in 1898* (M); Ft. Wingate, *Matthews* 68 (G). CHIHUAHUA: St. Diego, *Hartman* 601 (C, G, P); Chihuahua, *Palmer* 165 (US). HIDALGO: Buena Vista, *Pringle* 8929, type collection of *palustris* (C, G, US).

Oenothera flava is close to *O. triloba* and perhaps only a good variety, but it is certainly a distinct enough entity to merit a name. Its evident restriction to a different altitude and zone; its decidedly more perennial habit, its thicker leaves, etc. all argue for specific rank. Wootton & Standley's *Lavauxia hamata* has the wings of the capsule unusually well toothed and the tooth turns upward, but it does not seem to be more than a slight variation. Variation in *O. flava* in the amount of lobing in the leaves is very considerable; for example, such specimens as *Hitchcock* 1084 from Gold Creek, Nevada (US) and the *Nelson & Macbride* collection above cited from Mountain City, Nevada have almost no lobing; while a collection by *M. E. Jones in 1882* from Empire City, Nevada (P) and *MacDougal* 10 from Flagstaff, Ariz. (G) have very deep lobes.

3. *Oenothera taraxacoides* (Woot. & Standl.) Munz, n. comb.

Lavauxia taraxacoides Woot. & Standl., Contr. U. S. Nat. Herb. 16: 155. 1913.

Caudex short and thick, evidently perennial; leaves much as in *O. flava*, linear to oblanceolate in outline, bright green, 1-3 dm. long, 1-4 cm. wide, glabrate except for margins and veins, pinnatifid into narrow, acute, distant lobes, but not usually cut to near the midrib especially in the upper half of the leaf, and so passing insensibly into terminal lobe which is lanceolate, acute; flowers equaling or nearly as long as leaves; calyx-tube slender, 15-20 cm. long, quite glabrous without, finely pubescent within, gradually expanded at summit; calyx-lobes green to reddish, reflexed and adhering in anthesis, lance-linear, 35-40 mm. long, with free tips ca. 5 mm. long; petals yellow, obcordate, shallowly notched, 30-40 mm. long, 20-25 mm. wide; stamens somewhat unequal, the epipetalous filaments ca. one-third as long as petals, the alternate ones 3-4 mm. longer, all slightly flattened;

anthers linear, glabrous, 12–18 mm. long, the epipetalous ones slightly shorter than the alternate; style glabrous, about as long as stamens; stigma lobes linear, 6–8 mm. long; capsules ovoid-cylindric, indurated, 2–3 cm. long, with wings developed only toward the summit and 1–2 mm. wide, terminal teeth 1–2 mm. long; seeds as in *O. triloba*.

Type locality, James Canyon, Sacramento Mts., New Mexico. Representative material, NEW MEXICO: James Canyon, *Wooton*, July 6, 1899, type collection (P, US); White Mts., Lincoln Co., *Wooton* in 1899 (C, S, US); Cloudcroft, *Orcutt* 1336 (US); Tularosa Creek, Otero Co., *Wooton* in 1899 (US); White Mts., *Wooton* 664 (US). ARIZONA: Willow Spring, *Rothrock* 239 (G), *Palmer* in 1890 (C, G, US); White Mts., Apache Co., *Ferris* 1258 (S). CHIHUAHUA: Sierra Madre, *Nelson* 6047 (US), *Jones* in 1903 (P); Colonia Garcia, *Townsend & Barber* 48 (C, G, M, P); Madera, *Palmer* 324 (US).

Evidently related to *O. flava*, but larger flowered and with capsules less winged. The *Jones* collection from the Sierra Madre, Chihuahua has capsules practically without wings, but not at all resembling the wingless capsules of the subgenus *Pachylophus*.

MEGAPTERIUM

For the study of this subgenus I have had the herbarium material from Gray Herbarium (G), Pomona College (P), a few sheets from the collection Missouri Botanical Garden (M), and the material in the U. S. National Herbarium (US). I wish to express my appreciation to those who have made possible the use of this material and to Dr. I. M. Johnston of the Gray Herbarium for notes on literature and to Dr. F. Pennell of the Philadelphia Academy of Natural Sciences for photographs of Nuttall's specimens.

Subgenus **Megapterium** (Spach) Munz, n. comb.

Megapterium, as a genus, Spach, Hist. Veg. Phan. 4: 366. 1835; Raimann, in Engler & Prantl, Natürl. Pfl. fam. III, Abt. 7: 215. 1893; Small, Bull. Torrey Club 23: 183. 1896. *Megapterium*, as section, Endlicher, Gen. Pl., 1190. 1840.

Perennial, somewhat caulescent, the stems spreading. Leaves entire, to somewhat pinnatifid. Flowers vespertine, large, yellow, the calyx-tube much elongated. Stigma with 4 linear lobes. Capsule prismatic to ovoid, leathery or corky, more or less broadly winged and usually the entire length. Seeds as in *Lavauxia*, but in one row in each cell of the capsule, and with corky tubercles.

Type species, *Oenothera missouriensis* Sims.

KEY TO SPECIES

- Petals 18–22 mm. long; calyx-tube 25–45 mm. long; leaves subentire, lance-linear, 2–10 cm. long, strigose-canescens; capsule 3–4 mm. thick, with wings ca. 3 mm. wide. Kansas and Nebraska. 2. *O. Fremontii*.
 Petals 20–50 mm. long; calyx-tube 50–150 mm. long; leaves 4–20 cm. long, frequently pinnatifid, glabrous to hoary.

Wings of capsule 1.5-4.5 mm. wide; capsule 20-35 mm. long, narrowly ovoid to cylindric-ovoid; plants subacaulescent; leaves mostly pinnatifid.

Leaves thick, 10-30 mm. wide, subentire to many toothed; capsule wings not beaked nor toothed. Chihuahua to

Colorado and Idaho..... 3. *O. brachycarpa*.

Leaves thin, 2-6 (8) mm. wide, with few linear divaricate teeth, each capsule wing with terminal beak 3-5 mm. long and a spreading tooth 1-2 mm. long. Saltillo and Chihuahua..... 4. *O. graminifolia*.

Wings of capsule 7-20 mm. wide; capsule 20-65 mm. long, prismatic; plants with stems up to 5 dm. long; leaves mostly

entire. Kansas and Missouri to Texas..... 1. *O. missouriensis*.

TREATMENT OF SPECIES

1. *OENOTHERA MISSOURIENSIS* Sims, Bot. Mag., t. 1592. Nov. 1, 1813

Perennial, subacaulescent, to caulescent and 5 dm. tall, glabrous to hoary silky-strigose throughout; stems decumbent to erect, usually simple, often reddish; leaves linear-lanceolate to ovate or even obovate, acuminate to acute to obtuse, callous-tipped, subentire to remotely callose-denticulate, with more or less prominent marginal vein, the blades 3-10 cm. long, 0.5-3 cm. wide, narrowed gradually into petioles one-fourth to one-half as long and slightly winged; flowers few, very showy; calyx-tube 5-12 cm. long, ca. 2 mm. thick, somewhat enlarged at summit, glabrous to silky-strigose without, glabrous to puberulent within; calyx-lobes 2-4 cm. long, glabrous to silky, often with purplish spots, lance-linear, 4-5 mm. wide, with free tips 1.5-8 mm. long; petals yellow, drying yellowish to reddish, orbicular-obovate, subentire to serrulate on margin, with small terminal mucronate tooth 1-2 mm. long, which may be in a shallow sinus, petals 2-5 cm. long, equally wide; stamens subequal, ca. two-thirds the length of the petals; filaments somewhat flattened, quite dilated at base; anthers glabrous, linear, 12-22 mm. long; style about equal to petals, glabrous; stigma-lobes 5-7 mm. long; capsule prismatic, gradually tapering to summit, square in cross section, often reddish, glabrous to hoary, 4-7 mm. thick, 20-65 mm. long, indurate but hardly woody, with wings 7-20 mm. wide and often projecting slightly beyond the apex of the capsule, dehiscence terminal; seeds in 1 row in each cell, brownish to grayish, 3-4 mm. long, corky-tubercled, compressed, the wing 0.5 mm. wide, dentate and undulate.

KEY TO VARIETIES

Plant perfectly glabrous. Southern Kansas and

adjacent Oklahoma..... 1a. *O. missouriensis* var. *oklahomensis*.

Plant with more or less pubescence, either the stems and buds or the whole plant hoary. Kansas and Missouri to Texas.

Leaves linear-lanceolate to broadly lanceolate, greenish, with only the younger leaves, stems, etc., grayish..... 1b. *O. missouriensis* var. *typica*.

Leaves ovate to broadly lanceolate, hoary, the whole plant thickly invested with silvery

strigose pubescence..... 1c. *O. missouriensis* var. *incana*.

1a. *Oenothera missouriensis* var. *oklahomensis* (Norton) Munz, n. comb.

Megapterium oklahomense Norton, Rep. Mo. Bot. Gard. 9: 153. pl. 47, fig. 1-3. 1898. *Oenothera oklahomensis* A. S. Hitchcock, in Monde des Plantes 7: 148. 1898. *O. missouriensis* race *Nortoni* Leveille, Monogr. Onoth., 37. 1902.

Plant glabrous throughout; leaves lanceolate to oblong, 1-3 cm. wide; petals 3-4.5 cm. long; capsules 3-4.5 cm. long with wings up to 8 and 10 mm. wide.

Type locality, Marena, Oklahoma. Representative material, OKLAHOMA: Marena, type coll., *Waugh 183* (US); Avard, Woods Co., *Stevens 561* (G, US); Shattuck, Ellis Co., *Clifton 3169* (G); near Norman, *Bruner in 1924* (US); Zeigler, Major Co., *Stevens 596¾* (G). KANSAS: gypsum hills, Barber Co., *Hitchcock 165a* (G, US); Medicine Lodge, *Smyth 312* (US).

1b. *Oenothera missouriensis* Sims var. *typica* n. nom.

O. missouriensis Sims, Bot. Mag., t. 1592. Nov. 1, 1813; t. 1674, Oct. 1, 1814; Watson, Proc. Am. Acad. 8: 587. 1873; Leveille, Mon. Onoth., 33. 1902. *Megapterium missouriense* Spach, Hist. Veg. Phan. 4: 364. 1835; Small, Bull. Torrey Club 23: 184. 1896. *Oenothera macrocarpa* Nutt., in Fraser's Cat. no. 56. 1813; Pursh, Fl. Amer. Sept. 2: 734. 1814; Sweet, Brit. Fl. Garden, ser. 1, pl. 5. 1823. *Megapterium macrocarpum* Gates, Ann. Mo. Bot. Gard. 1: 402. 1915. *Oenothera alata* Nutt., Gen. N. Am. 1: 248. 1818. *Megapterium Nuttallianum* Spach, l.c. *Oenothera missouriensis* var. *latifolia* Gray, Bost. Journ. Soc. Nat. Hist. 6: 188. 1850. *O. missouriensis* f. *intermedia* Levl., Mon. Onoth., 36, pl. 5. 1902.

Stems and buds hoary-strigose, but leaves green; leaves lanceolate to linear-lanceolate.

Type locality. "Found by Mr. Nuttall in the neighborhood of the Missouri." Ranging into western Kansas and south to Texas. Representative material, MISSOURI: St. Louis, Nuttall, type material of *macrocarpa*, photograph of specimens in Philadelphia Acad. (P); Swan, *Bush 172* (G, US); Morley, Franklin Co., *Letterman in 1883* (US); De Soto, Jefferson Co., *Eggert in 1896* (US); Cedar Gap, Ozark Mts., *Lansing 3045* (G, US). KANSAS: Manhattan, *Dice in 1917* (US). OKLAHOMA: on the False Wichita, *Palmer 131* (US). TEXAS: Ft. Worth, *Ruth 34* (G); Dallas, *Reverchon 905* (G, P, US), *E. Hall 207*, type coll. f. *intermedia* (G, P, US); Kerrville, *Heller 1629* (G, P, US); New Braunfels, *Munz 1288* (P); Spanish Pass, Kendall Co., *Clemens in 1911* (P); Helotes, Bexar Co., *Schulz 257* (US).

If we accept the short description of *O. macrocarpa* in Fraser's Catalogue as sufficient to constitute publication, it may antedate that of *O. missouriensis*, but I have been unable to find out the exact date of the former. Gray's var. *latifolia* is merely a new name for *macrocarpa*; so far as I can see, however, there is no justification for dividing the plants from Missouri

into a narrow-leaved or typical variety and a broad-leaved one. Apparently both *missouriensis* and *macrocarpa* came from the same general region; so far as I can discover, the differences shown in plates, descriptions, and the like are normal variations that do not require taxonomic recognition.

1c. *OENOTHERA MISSOURIENSIS* var. *INCANA* Gray, Boston Journ.
Nat. Hist. 6: 189. 1850

Megapterium argyrophyllum Gates, Ann. Mo. Bot. Garden 1: 401, pl. 23.
1915. *M. argyrophyllum* var. *retusifolium* Gates, l.c.

Silky-hoary throughout; leaves ovate to ovate-lanceolate; capsules with wings 1.5–2 cm. wide.

Type locality, on the Canadian River, Kansas. Representative material, KANSAS: on the Canadian, *Gordon 31*, type (G); Greensburg, *Smyth 89* (US); Riley Co., *Norton 163a* (G, US); Osborne City, *Shear 29* (G, US); Manhattan, *Dorman in 1893* (US). OKLAHOMA: Cache, Comanche Co., *Stevens 1346 1/2* (G); Shattuck, Ellis Co., *Clifton 3173* (G). TEXAS: Hereford, Deaf Smith Co., *Osterhout 34* (US); Canon City, *Reverchon 3839* (G, US); Amarillo Creek, *Reverchon 2749*, type coll. *argyrophyllum* and var. *retusifolium* (US); Comanche Plains, *Bigelow in 1853–54* (US); Rio Grande Valley, *Bigelow 362* (US).

There is of course intergradation between this variety and *typica*, some specimens such as *Norton 163a* being quite intermediate in pubescence. I have rather arbitrarily designated *Gordon 31* as the type. Gray in his original description gives "on the upper Platte and Canadian, Fremont and Mr. Gordon." At the Gray Herbarium there are two Fremont specimens collected in 1845, one quite like *O. Fremontii*. The Gordon collection is perfectly representative of *incana* and to select it as the type avoids any possible confusion.

2. *OENOTHERA FREMONTII* S. Wats., Proc. Am. Acad. 8:587. 1873

Megapterium Fremontii Britton, Mem. Torrey Club 5: 236. 1894; Small,
Bull. Torrey Club 23: 183. 1896. *Oenothera missouriensis* race
Fremontii Leveille, Mon. Onoth., 36. 1902.

Subcaulescent perennial, tufted, strigose-canescens throughout, with the pubescence very dense, short and silky; caudex woody and few-branched; stems 2–10 cm. long; leaves lance-linear, the blades 2–6 cm. long, 2–3 (5) mm. wide, callose-margined and -tipped, subentire to remotely callose-denticulate, acuminate, gradually narrowed at base into flattened petiole 0.5–3 cm. long; flowers few, axillary, vespertine; calyx-tube slender, strigose, 2.5–4.5 cm. long, puberulent within; calyx-lobes united in anthesis, lance-linear, 20–25 mm. long, ca. 3 mm. wide, canescent, with free tips ca. 1 mm. long; petals yellow, orbicular-obovate, 18–22 mm. long, with irregular margin, emarginate with short tooth at end of mid-vein; stamens subequal, almost as long as petals; filaments somewhat flattened, 10–15 mm. long; anthers 6–10 mm. long, glabrous; style equaling or slightly exceeding petals,

glabrous; stigma-lobes 3-4 mm. long; capsule 15-25 mm. long, square in cross-section, 3-4 mm. thick, prismatic, with wings ca. 3 mm. wide, these not projecting beyond the capsule; ripe seeds not seen, immature ones light brown, 2 mm. long, corky-tubercled and apparently with terminal toothed wing, seeds perhaps 8-10 in each cell.

Type locality, probably in Kansas, type collected on the second Fremont Expedition. Representative material, KANSAS: Stockton, Rooks Co., *Kellogg in 1911* (US); Cliffs, Rooks Co., *Popenoe in 1875* (US); Medicine Lodge, *Keltermann in 1888* (US); stony hills, Russell Co., *A. S. Hitchcock 165* (G, US); Barber Co., *Carleton in 1888* (P); Smoky Hill, *Parry in 1867* (G). NEBRASKA: Red Cloud, *Bates 2889* (G). Apparently rather a rare species and one approaching *O. brachycarpa* and *O. missouriensis*. For example, *Hitchcock 165* has wings of capsules unusually broad, suggesting *O. missouriensis*. *Bates 2889* is more glabrous than most other collections and with the petals 25 mm. long, suggesting the same species. The *Kellogg* collection above cited has two plants with leaves suggesting *O. brachycarpa* in shape and denticulation, but with the pubescence and flower size of *Fremontii*.

3. OENOTHERA BRACHYCARPA A. Gray, Pl. Wright. 1: 70. 1852

Subcaulescent perennial, with a heavy simple or branched caudex or with stems occasionally up to 1 dm. long; closely cinereous-strigillose, or even hoary, throughout; leaves tufted, thick in texture, the blades linear to lance-ovate in outline, 3-15 cm. long, 1-3 cm. wide, varying from almost simple, subovate, and irregularly crenate-dentate, 1-5 cm. long with petioles of about same length, to longer, with few minute or larger lateral lobes or teeth, to lance-linear and subentire with petioles much shorter than blades, to having a linear, acuminate, entire terminal portion ca. 1 dm. long and with few, sinuate, divaricate, linear lateral lobes and gradually narrowed below into winged petiole 5-6 cm. long; flowers large, yellow, vespertine, with but one or two in anthesis at once; calyx-tube 5-15 cm. long, strigillose-canescens without, finely pubescent within, gradually ampliate toward the summit; calyx-lobes lance-linear, 3-4 cm. long, 3-4 mm. wide, often tinged purplish, strigillose-canescens to hoary, with free tips 1-4 mm. long; petals orbicular-obovate, 3-5 cm. long, with or without shallow terminal sinus, yellow, drying reddish; stamens subequal, ca. two-thirds the length of the petals, filaments somewhat flattened, ca. 1 mm. wide; anthers 10-12 mm. long; style glabrous, equaling stamens or even petals; stigma-lobes 5-10 mm. long; capsules 2-3.5 cm. long, 6-8 mm. thick, ovoid to cylindric-ovoid, with walls varying from 0.5 to 1.5 mm. thick, winged only near the summit or almost the entire length; wings 1.5-4.5 mm. wide, not ending in free teeth; seeds dark brown, 2-4 mm. long, corky-tubercled or merely roughened toward the base with corky layer, the summit with denticulate corky wing.

KEY TO VARIETIES

- Leaves narrow, the long terminal portion of blade 3-6 mm. wide, with few pairs of remote spreading narrow lobes; leaves not pinnatifid to midrib; capsule ca. 2 cm. long. West central Texas. 3a. *O. brachycarpa* var. *typica*.
 Leaves broader, the terminal portion of blade generally 1 cm. or more wide, the lower portion entire or

variously pinnatifid or lobed; capsule 2-3.5 cm. long.

Western Texas and Chihuahua to Colorado, Idaho,

and Nevada. 3b. *O. brachycarpa* var. *Wrightii*.

3a. *OENOTHERA BRACHYCARPA* A. Gray var. *TYPICA* n. nom.

O. brachycarpa Gray, l.c.; Wats., Proc. Am. Acad. 8: 586. 1873. *Lavauxia brachycarpa* Britton, Mem. Torr. Bot. Club 5: 235. 1894; Small, Bull. Torrey Club 23: 182. 1896. *Megapterium brachycarpum* Leveille, Mon. Onoth., 39. 1902, in synonymy.

Leaves narrow, the terminal portion of the blade 3-6 mm. wide, 3-10 cm. long, subentire or remotely denticulate, acuminate to acute, the lower portion remotely, shallowly and sinuately dentate or pinnatifid, gradually narrowed below into a winged petiole; capsule ovoid, ca. 2 cm. long.

Type locality, western Texas. Material seen, TEXAS: "Western Texas to El Paso, *Wright in 1849*, type (G); Baird, Callahan Co., *Reverchon 301* (M); Sweetwater, *Reverchon 52* (G); Belknap, *Hayes 259* (G). As here treated *O. brachycarpa* var. *typica* is a rare and but seldom collected plant. There has been much confusion concerning *brachycarpa*; in fact, the original description is confusing, since it is based on two quite different collections both by C. Wright. I am taking as the type, the unnumbered 1849 collection which is the one designated by Gray as "Between Western Texas and El Paso; in fruit." The 1851 collection of which he speaks and which is mounted on the same sheet, is *Wright 1073* from New Mexico and is the broad-leaved plant that I refer to var. *Wrightii*. The use of these two collections in making up his description explains Gray's speaking of the earlier leaves as being entire to repand, etc. and the succeeding ones narrower. The type has the capsule very slightly winged (2 mm. wide in upper part only) but the other collections I have above cited as belonging to *typica* have the wings the entire length and wider.

3b. *OENOTHERA BRACHYCARPA* var. *WRIGHTII* (A. Gray) Leveille,
Mon. Onoth., 40. 1902

O. Wrightii A. Gray, Pl. Wright. 2: 57. 1853; Watson, Proc. Am. Acad. 8: 586. 1873. *O. Wrightii* var. of Gray, l.c. *Lavauxia Wrightii* Small, Bull. Torrey Club 23: 183. 1896. *Oenothera Howardi* M. E. Jones, Zoe 3: 301. 1893. *Lavauxia Howardi* A. Nels., Bot. Gaz. 34: 368. 1902.

Leaves broader, variable, the blades 8-35 mm. wide, oblanceolate to ovate, greenish or hoary, entire to sinuate-pinnatifid or even with a few lateral lobes; capsule longer than in *typica*, 2.5-3 cm. long.

Type locality, near the "Copper Mines," now Santa Rita, Grant Co., New Mexico. Ranging at a higher altitude and far to the north and west of var. *typica*. Representative material, TEXAS: Davis Mts., *Ferris & Duncan 2639* (S); El Paso, *Carlson in 1915* (G); Limpia Canyon, *Tracy & Earle 291* (US). NEW MEXICO: *Wright 1072*, type coll. (G, US); Cloud-

croft, *Schulz* 276 (US); San Andreas Mts., *Wootton in 1913* (US); Big Hatchet Mts., Grant Co., *Mearns* 50 (S, US). CHIHUAHUA: Sierra Madre, *Jones in 1903* (P); St. Diego Ranch, *Hartman* 675 (G). DURANGO: Tepehuanes, *Palmer* 46 (US); Durango, *Palmer* 277 (G). ARIZONA: Santa Rita Mts., *Pringle in 1884* (US); Chiricahua Mts., *Blumer V* 171 (US). COLORADO: Arvada, *Clokey* 4221 (P, S); Morrison, *Eastwood in 1891* (G); Boulder, *Vestal in 1913* (S); Ft. Collins, *Baker in 1894* (P). UTAH: San Rafael Swell, *Jones in 1914* (P); Vermilion, *Jones* 5631 o (P); Dugway, Tooele Co., *Jones in 1891*, type coll. of *Howardi* (G, P); Deep Creek, *Jones in 1891* (P). NEVADA: Charleston Mts., *Jones in 1927* (P), *Heller* 10999 (G, S); Comet Peak, Pioche, *Jones in 1912* (P); Pahrangat Mts., *Searls in 1871* (G). IDAHO: Weiser, *Jones in 1900* (P).

Wrightii is a broader-leafed and for the most part more hoary plant than is *typica* and in its extremes would seem to be worthy of specific rank, being much showier as to flower. However such specimens as the *Baker* collection from Ft. Collins, Colo. and the *Vestal* one from Boulder are quite intermediate. Nor do the more western plants, for which the name *Howardi* has been proposed, offer any constant enough differences to merit naming. There are perhaps the following very poorly defined tendencies: (1) plants from the El Paso region are apt to be hoary, with leaves short (4–6 cm. long), and with ovate terminal lobe, (2) those from Colorado and southern Utah more green, with leaves narrower and longer (10–20 cm.) and the terminal lobe lanceolate, (3) those from western Utah and Nevada hoary, with leaves 1–2 dm. long, and the terminal lobe lanceolate.

4. OENOTHERA GRAMINIFOLIA L'éveillé, Mon. Onoth., 42, pl. 3. 1902

Lavauxia graminifolia Rose, Contr. U. S. Nat. Herb. 8: 329. 1905.

Oenothera brachycarpa var. *stenophylla* Levl., l.c., published in synonymy.

Perennial, subcaulescent or with few short spreading stems up to 1.5 dm. long, these glabrate to strigillose, arising from a branching woody caudex; leaves thin, more or less tufted, linear, the blades 8–15 cm. long, 0.5–2 cm. wide, sparingly strigillose, subentire and 2–4 mm. wide, or even 6–8 mm. wide and with a few linear divaricate teeth and gradually narrowed into winged petioles ca. one-third their length; calyx-tube and -lobes hoary-strigillose, the former slender, 10–15 cm. long, finely pubescent within, gradually and slightly enlarged at summit; calyx-lobes lance-linear, adhering in anthesis, 25–28 mm. long, 4–5 mm. wide, with free tips only ca. 1 mm. long; petals yellow, drying pinkish, suborbicular-obovate, 33–40 mm. long, without evident sinus; stamens subequal, filaments flattened, 2–2.2 cm. long; anthers 11–13 mm. long, glabrous; stigma-lobes linear, 4–6 mm. long; capsule narrowly ovoid, 2–3 cm. long, 5–6 mm. thick, with terminal beak 3–5 mm. long and spreading teeth 1–2 mm. long; seeds dark brown, corky tubercled, 3 mm. long, with wing at summit toothed.

Type locality, Saltillo, Coahuila, Mexico. Material seen, COAHUILA: Saltillo, *Palmer 342*, type coll. (G, M, US), cited as 14,322 by Leveille, the sheet at Missouri Bot. Gard. bears both numbers; Saltillo, *Palmer 2120* (G). CHIHUAHUA: Ojo Caliente, *Gregg 129* (M). *Palmer 2120* has wider, more lobed leaves than the others and approaches *O. brachycarpa* var. *typica*. In fact, *O. graminifolia* might be included as a variety in *O. brachycarpa* to which it has evident affinity. The spreading terminal teeth of the capsule and the thinner more delicate leaves which are less pubescent than in *O. brachycarpa* make it quite intermediate between that species and *O. triloba*.

POMONA COLLEGE,
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THE ENDOSPERM OF *ZEa* AND *COIX*¹

PAUL WEATHERWAX

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The endosperm of the cereal grasses has been the subject of numerous investigations directed from many different points of view, but certain phases of its development, involving the differentiation of its tissues, the accumulation of food material, and the relations between the endosperm and the embryo, have not been thoroughly studied.

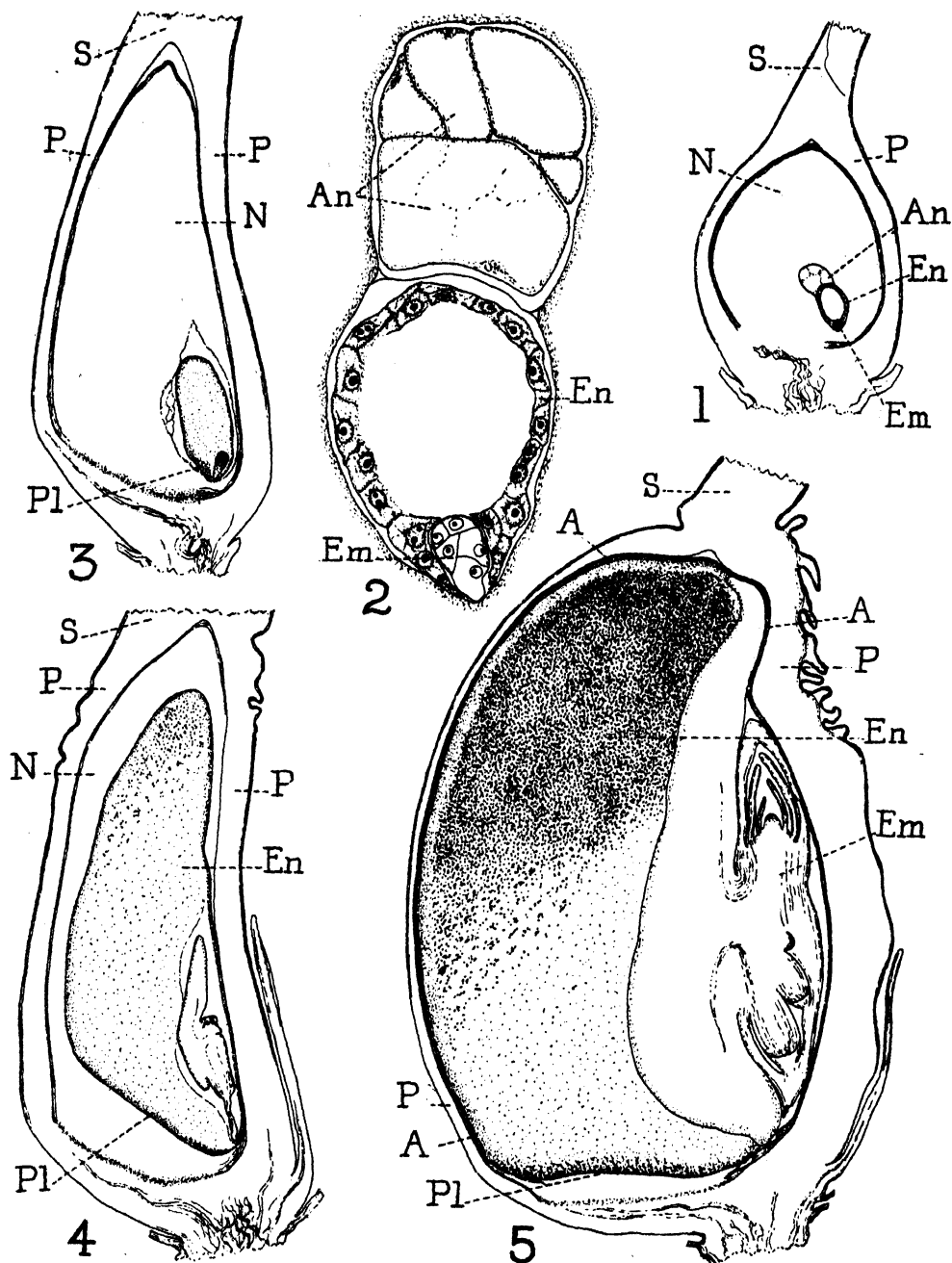
The application of the principle of the alternation of generations to the higher plants during the latter part of the past century gave to the endosperm a new morphological meaning, which was modified and made more interesting by the discovery of "double fecundation" and by the studies on xenia at the very close of the century. In most studies of development, however, the embryo has been the structure emphasized, and the chief interest in the endosperm has been its rôle as a nurse tissue for the embryo.

From the work that has been done on various grasses it is evident that there is considerable uniformity in the development of the endosperm. The polar nuclei remain in contact with each other, without fusing, until the time of fecundation. The union of a sperm with them has been observed in *Zea* (9, 12, 17) and in *Triticum* (11), and all circumstantial evidences indicate that double fecundation regularly occurs in other species.

After fecundation there is a period of rapid division of free nuclei, with a migration of nuclei toward the antipodal end of the endosperm cell (Randolph, 13). These nuclei are located in a thin wall layer of cytoplasm, and the center of the cell is a large vacuole (Pl. XXVIII, fig. 13). The formation of cell walls apparently begins in the peripheral region, for a stage of development is soon to be seen in which the endosperm is a hollow mass of tissue surrounding a central cavity (text figs. 1, 2). The steps of development preceding this stage have not been investigated in detail, but it is reasonable to assume that they are similar to those of corresponding stages in the development of the endosperm of typical angiosperms. It would probably be better to regard this hollow endosperm as a cellular mass having a large, multinucleate cell in the middle. This cavity is soon eliminated by further nuclear division and wall formation (text fig. 3 and Pl. XXVIII, fig. 14).

Of the course of development of the endosperm from this time onward we have only fragmentary information drawn from studies of different grasses. These studies long ago indicated considerable differentiation in the mature endosperm of the cereals.

¹ Publication 46 of the Waterman Institute, Indiana University.



TEXT FIGS. 1-5. Longitudinal sections of the caryopsis of *Coix* showing successive steps in the development of the endosperm. FIG. 1. At the time of the beginning of cell formation in the endosperm. FIG. 2. Endosperm and embryo of the same seed. FIG. 3. Formation of cell walls has extended to the middle of the endosperm. FIG. 4. Embryo differentiating. FIG. 5. Seed almost mature. A, aleurone layer; An, antipodal tissue; Em, embryo; En, endosperm; N, nucellus; P, pericarp; Pl, placental tissue of endosperm; S, base of style. (FIG. 2, $\times 160$; others, $\times 20$.)

A peripheral layer, one cell thick (or occasionally thicker), is known as the aleurone layer because of its high aleurone content. Miss Gordon (7) pictures the aleurone layer of the mature grain as a resting cambium which has been responsible for most of the cell divisions following the free-nuclear stage; another investigator is reported to have attributed this cambial property to the cells immediately below the aleurone layer.

Differentiation of the portions of the endosperm below the aleurone layer, on the basis of hardness, color, chemical nature, etc., has been investigated in many ways, and it is generally conceded that these differences can be explained in terms of stored food materials.

Several studies have attempted to trace the development of plastids and the manner of deposition of food, and a few have investigated chromo-some numbers and other things related to cell division.

Some significant points not thus far reported in connection with the development of the endosperm have been found in a series of sections of grains of *Zea Mays* made several years ago in tracing the development of the embryo. This material has been supplemented with sections of the developing seed of *Coix lacryma-jobi*. The general course of development is the same in both species, but a comparison of the two, and the use of one to fill in places where the other is not clear, has led to a better understanding of both. Where statements are made without mention of the genus they are intended to apply to both of these genera in so far as my investigations have gone. The figures are all of *Coix*, except figure 13, Plate XXVIII.

The variety of *Coix* studied is the one often grown in gardens for its fruits, which are used for beads and commonly known as "Job's tears." Several varieties of maize were used, but they all tell the same story, with minor variations where the mature structure of the seed is concerned. The material was fixed in chromo-osmo-acetic acid or chromo-acetic acid of various strengths and imbedded, sectioned, and stained in the usual ways. Sections of parts of mature seeds were also made without imbedding.

The observations here recorded and the conclusions drawn from them are concerned chiefly with the following points: (1) the parasitic relation of the embryo to the endosperm; (2) the source of food supply of the endosperm and its method of absorbing food; (3) the competition of tissue systems in the developing seed; (4) the manner of deposition of food in the endosperm; (5) differentiation in the endosperm; and (6) the bearing of some of these points upon the question of the individuality of the endosperm.

RELATION OF EMBRYO TO ENDOSPERM

At the time when the formation of cell walls has proceeded to the middle of the endosperm, eliminating the central cavity (text fig. 3 and Pl. XXVIII, fig. 14), the embryo is a small, oval or pyriform body near the micropyle. Although located well toward the side facing the lemma, the

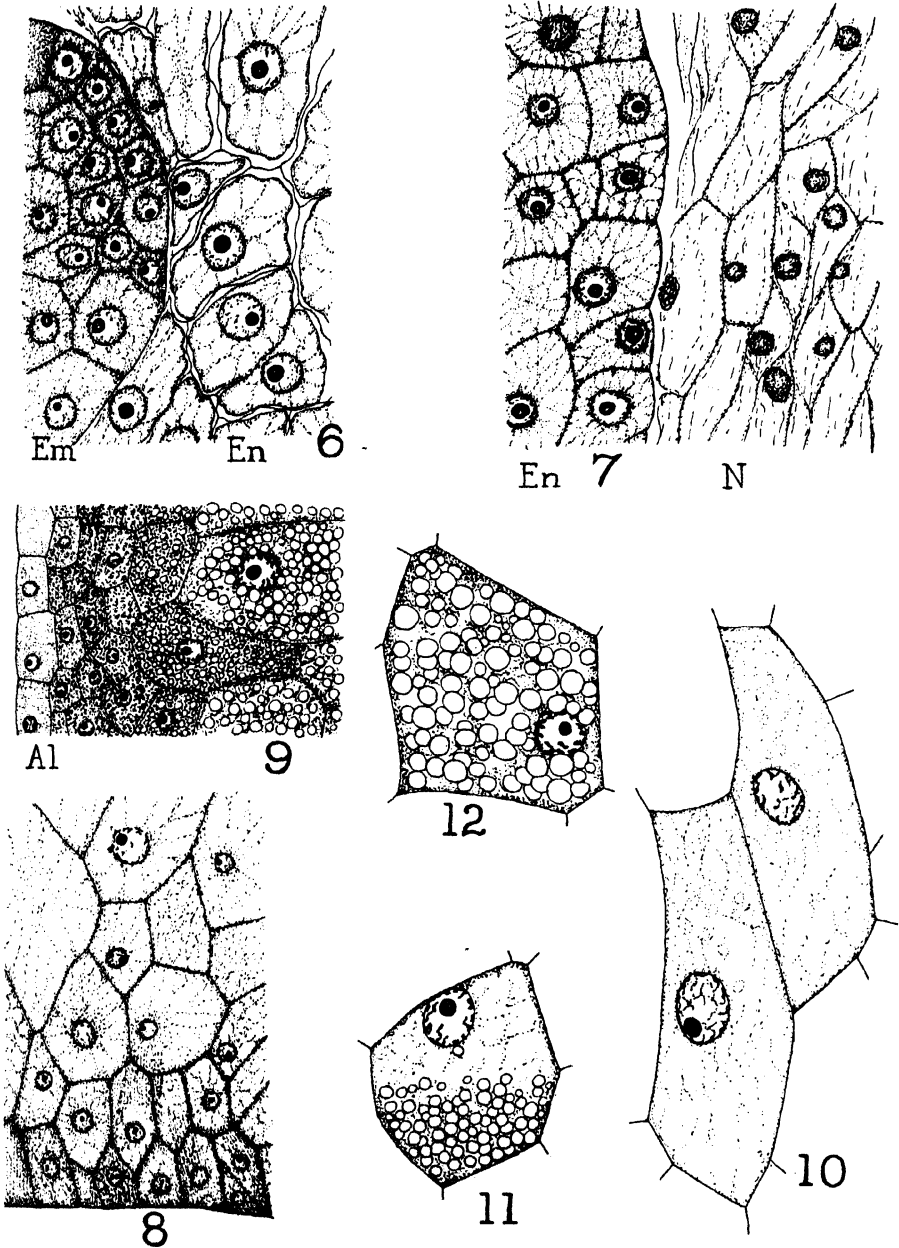
embryo is at this time completely surrounded by the endosperm except at its base where the suspensor extends to the outside. When the seed is mature the embryo is apparently imbedded in the side of the endosperm, its outer surface being covered only by the pericarp and the remnant of the inner integument. Sections made at different times during development show, however, that a thin layer of the endosperm persists on the outer surface (text figs. 4, 5). Continued growth by cell division in this layer seems to enable the endosperm to hold its own against the destructive effect of the embryo in this direction in the earlier stages of development. Later, when the parts of the embryo have become differentiated, it seems probable that the young coleoptile and coleorhiza, with the folds of the cotyledon which partly cover them, are less active in digesting and absorbing the endosperm than is the scutellum, for few disorganized cells and few dividing cells are to be seen next to these organs as the seed approaches maturity. At maturity, as a rule, at least the aleurone layer remains on the outer side of the embryo (text fig. 5).

The nutrition of the embryo presents some problems for the solution of which full data are not yet available. That it digests cells of the endosperm is not to be doubted, because there is always to be seen a layer of partly disorganized cells in contact with it; but this is not a sufficient explanation of the source of its food. At least in early stages of development, the endosperm cells which are consumed contain at any one time very little food, while the adjacent cells of the embryo have the appearance of being well nourished (text fig. 6 and Pl. XXVIII, figs. 14, 15). It follows that the cells of the endosperm next to the embryo must have some sort of vascular function, transporting food from other parts of the endosperm to the layer of disorganizing cells, where it is absorbed by the embryo.

NUTRITION OF THE ENDOSPERM

As the endosperm increases in size the nucellus is destroyed, and none of it remains at maturity. The mechanism of this destruction of tissue is not known. Haberlandt (10, pp. 458-460) establishes the fact that the aleurone layer produces enzymes during germination, and it seems reasonable that its forerunner here in the surface layer of the endosperm may do the same thing.

Any theory to the effect that the endosperm nourishes itself to any considerable degree by merely consuming the nucellus has the same defects as were pointed out in the relations between the embryo and the endosperm. The large, vacuolate cells of the nucellus, with their small nuclei and attenuated cytoplasm, could not furnish enough food to supply the growing endosperm (text fig. 7 and Pl. XXVIII, figs. 14, 16). Moreover, the nucellus has been completely destroyed before any considerable amount of food has been permanently deposited in the cells of the endosperm, and the greater portion of the rich supply present at maturity must be brought in after the complete disappearance of the nucellus.



TEXT FIGS. 6-12. Details of embryo and endosperm of *Coix*. FIG. 6. Contact between young embryo, *Em*, and endosperm, *En* ($\times 600$). FIG. 7. Contact between endosperm, *En*, and nucellus, *N* ($\times 600$). FIG. 8. Placental tissue at base of endosperm shown in Figure 5 ($\times 180$). FIG. 9. Aleurone layer, *Al*, and adjacent tissue of the same endosperm ($\times 180$). FIG. 10. Cells from region just above placental tissue, serving, at least temporarily, a vascular function ($\times 180$). FIG. 11. Cell, partly filled with starch, from near middle of same endosperm ($\times 180$). FIG. 12. Cell from near top of same endosperm ($\times 180$).

An answer to the question suggested is found in a special tissue at the base of the endosperm (Pl in text figs. 3-5, and Pl. XXVIII, fig. 15). Its structure and position from the first suggest a special function. Its cells are relatively small, and usually elongated and angular in shape (text fig. 8). The nuclei and dense cytoplasm have the appearance of an active living condition, and karyokinetic figures are frequent. It is also in the position nearest the vascular complex which supplies the ovule. These facts indicate that this specialized region has a placental function, taking food from the source of supply and passing it on to other parts of the endosperm. This theory is further supported by the position of this tissue relative to the order of deposition of food in the endosperm.

COMPETITION OF TISSUE SYSTEMS

As the writer has pointed out in another study (18) there are in the developing fruit of a grass four individual structures which are in a more or less antagonistic relation to one another. They are: (1) the parental sporophyte, represented by the nucellus and the integuments; (2) the filial sporophyte, the embryo of the seed; (3) the gametophyte, represented by the antipodal tissue; and (4) the endosperm.

In the ordinary course of events the antipodal tissue disappears soon after fecundation in both *Zea* and *Coix*. Sometimes, however, as the writer has reported (18), its cells may continue to multiply, enabling the tissue to persist until the seed is mature. Except possibly in rare genetic monstrosities the endosperm always destroys the nucellus, and it is in turn destroyed by the embryo, partly before maturity of the seed and partly during germination. In each of these instances what determines which structure shall survive? What we know of enzymes and their various activators and protectors indicates that to answer this question we must do a great deal of careful microchemical work. For the present we can only speculate.

The endosperm presents a strange paradox. Its vigor and its parasitic relation to the nucellus might be attributed to its triploid chromosomal constitution, but this could scarcely explain at the same time the certainty with which it succumbs to another diploid generation, the embryo.

The problem might be attacked from the standpoint of permeability, for, whenever the border zone is examined, the cells of the invading tissue are found to be turgid, while those of the tissue that is being destroyed often show more or less plasmolysis before actual disintegration begins (text figs. 6, 7 and Pl. XXVIII, figs. 13-15). Until the cause of this difference in permeability is established, however, we are still almost as far as ever from an answer to the question.

The technique of recent studies on differentiation and senility promises results when applied to the problem. When the endosperm begins to develop, the nucellus is an old and differentiated tissue. Its cells have

ceased active division, their protoplasm is attenuated, and they have developed no thickened walls or other devices that might protect them. In contrast with this, the embryo is at first almost wholly meristematic. Later, when a degree of differentiation has taken place, it presents a glandular surface to the endosperm (6, 14).

Between these two sporophytic tissues is the endosperm, itself a differentiated structure. Its periphery, in contact with the senile tissues of the ovary (text fig. 7), is meristematic; but it presents to the vigorous young embryo an old and practically mature tissue of internal derivation (text fig. 6). The persistence of the thin layer, consisting of the aleurone layer and probably a few other cells, over the face of the embryo (text figs. 4, 5) is probably due as much to the perpetual youth of this tissue as to the inefficiency of that side of the embryo as an agency of digestion.

DEPOSITION OF FOOD IN THE ENDOSPERM

As has previously been stated, most of the food used by the developing endosperm probably comes in through the placental region at the base. In early stages of development this is used for growth, but usually, by the time the endosperm is half grown, starch grains and protein granules make their appearance in the cells toward the upper part.

The protein granules are usually most numerous in the meristematic cells near the surface (text fig. 9), becoming fewer toward the center of the endosperm. Starch grains, on the other hand, begin to develop at some distance from the surface in cells which have reached practically their full size. The formation of starch continues downward through the endosperm, its pattern of distribution being directed largely by hereditary constitution. As the seed approaches maturity the region of starch deposition moves outward toward the periphery until the fully matured seed has only the aleurone layer free of starch. A short time before maturity (text fig. 9) one or more layers of cells beneath the aleurone layer are still free of starch, and this might lead to the assumption that the aleurone is more than one cell in thickness. There is a slight difference in appearance, however, between the aleurone cells and those lying immediately below them. This seems to be due to the size of the protein granules, those of the aleurone cells being smaller.

As the starch grains are formed in any one cell they tend to settle to the bottom. Consequently, all partly filled cells in any one endosperm show the starch grains clustered in the same side (text fig. 11), depending upon the position in which the seed stood on the plant.

DIFFERENTIATION IN THE ENDOSPERM

The endosperm shows, during development and at maturity, a higher degree of differentiation than is usually attributed to it. As has been stated, the peripheral portion is meristematic and cells more deeply located

illustrate very well the stages of elongation and maturation usually found in a developing organ. Maturation probably consists here of the processes resulting in the storage of food (text fig. 12).

The placental nature of the basal portion (text fig. 8) has been pointed out, and certain elongated cells more deeply located (text fig. 10) doubtless serve for a time a primitive vascular function, carrying the food in solution from where it enters the endosperm to the upper parts where storage begins.

The exact pattern followed in the storage of starch in any individual seed is determined largely by genetic factors and it governs the extent, distribution, and texture of the flinty and floury portions of the endosperm so evident in the varieties of *Zea*, and less so in *Coix*.

INDIVIDUALITY OF THE ENDOSPERM

Following the discovery of "double fecundation" in angiosperms, in 1899, there ensued a long controversy as to the sexual or non-sexual nature of this fusion of three nuclei and as to the phylogenetic significance of the endosperm. Strasburger (16), DeVries (5), Guignard (8, 9), Sargent (15), Berridge (1), Coulter (4), Correns (2), and many others have discussed the question from various theoretical angles. Strasburger, in particular, has opposed the idea that this nuclear fusion is sexual. Others have been more inclined to regard it as sexual and to place the endosperm on much the same plane as the embryo, except that its fate is sealed from the beginning and it leaves no descendants.

It is certainly not wise at present to attempt to explain the endosperm of all angiosperms by means of one theory, for there is much variation in its origin and development in the different families. It is futile also to argue the question of its sexual or non-sexual origin even in the grasses, where there is indication of much uniformity, without some generally accepted definition of sexuality.

As the situation stands at present, however, the greater part of Strasburger's argument has been turned against his thesis, at least as far as such genera as *Zea* is concerned, for the nuclear fusion giving rise to the endosperm complies with most of his requirements for a true sexual union. The stimulus to development is certainly given; the work of numerous investigators on xenia establishes the fact that hereditary potentialities are transmitted; and the orderly development and relatively high degree of differentiation in the endosperm give it many of the characteristics of an individual plant. It probably fails to fulfill the complete requirement in the fact that it is always destroyed by the embryo and, consequently, does not reproduce. The latter deficiency may be attributed, tentatively, at least, to its triploid chromosomal constitution, and its mechanism may be the premature differentiation of the portion of the endosperm in contact with the embryo. The experimental demonstration of the validity of these

surmises must be left to a better experimental technique than has thus far been applied to the problem.

SUMMARY

The embryo in *Zea* and *Coix* is embedded in one side of the endosperm, but surrounded by the latter except at the base. At maturity of the seed the outer surface of the embryo is covered by at least the aleurone layer of the endosperm.

The peculiar nutritive relations of the endosperm, parasitic on the maternal and host to the filial sporophyte generation, is probably best explained in terms of differentiation and senility. The older and more differentiated tissue in each case gives way to the tissue which has retained its meristematic character.

Nutrition of the endosperm is accomplished chiefly by a placental tissue at its base near the point of entrance of the vascular supply of the ovary, rather than by absorption from the nucellus.

Deposition of food begins at the top of the endosperm and proceeds downward, following a hereditary pattern. This pattern, together with environmental factors, determines the final texture of the endosperm.

The endosperm has a definite ontogeny, and it shows at maturity a higher degree of differentiation than is ordinarily attributed to it. These facts strengthen the theory that the endosperm is to be regarded as an individual, a definite generation in the life history of the plant, rather than merely a nurse tissue for the embryo.

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DESCRIPTION OF PLATE XXVIII

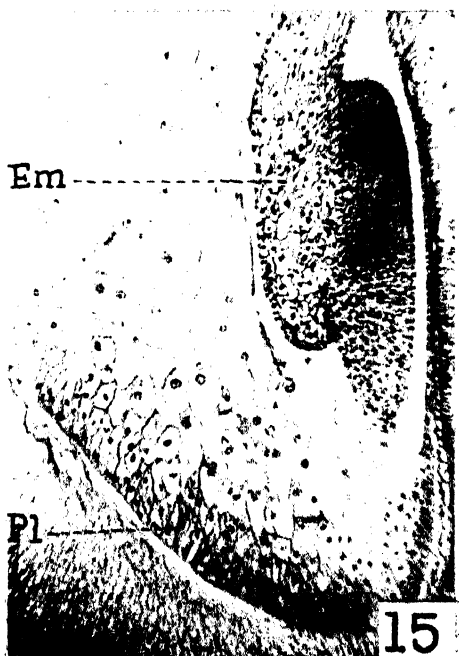
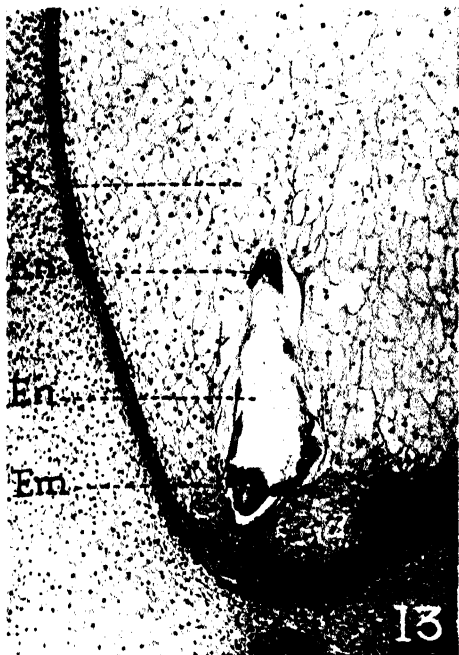
FIG. 13. Embryo sac of *Zea* soon after fecundation; free-nuclear stage of endosperm development ($\times 70$).

FIG. 14. Young embryo and endosperm of *Coix* ($\times 70$).

FIG. 15. Older embryo of *Coix* with cotyledon in contact with endosperm ($\times 70$).

FIG. 16. Endosperm of same seed in contact with nucellus ($\times 70$).

Legends as in text figures 1-12.



THE EFFECT OF METALS ON THE RESPIRATION OF *LUPINUS ALBUS*

WILLIAM CHRISTIAN LEVAN

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INTRODUCTION

It has been known for many years that some toxic substances, at proper concentration, cause a stimulation of carbon dioxide production in plants. Both inorganic and organic compounds, including anesthetics, have been studied in their effect upon lower organisms as well as upon higher plants. The present investigation is undertaken to determine the effect of certain representative metals in the form of usual salts on the respiration of a seed plant, *Lupinus albus* L. It was found in preliminary experiments that absorption through the root system proceeded slowly and with consequent complications. With this fact in view, a study is made to learn the effect of solutions of the salts of these metals when taken directly into the cut stem and up into the plant with the ascending solution stream and not through any external absorbing surface. It is believed that by this process the salts reach the cells of the leaves more quickly, and with little change in concentration. By this method, the object of the investigation is to discover the range of concentration that stimulates CO₂ production, the length of time the stimulation is effective, the percent of increase in respiration, and the relation of the rates of respiration produced by the several metals employed.

HISTORICAL REVIEW

Apparently the solutions of many metals affect the CO₂ production of plants either by causing an increase in rate followed by a decrease or by proving to be so toxic that a decrease prevails from the beginning. Cook (7) investigated the effects of Cu, Hg, Ag, and the H-ion concentration on the respiration of *Aspergillus niger*, and found a decrease in the rate of CO₂ production from the first or an increase and then a decrease. He states that the toxic action varies as a constant power of the concentration. In another paper the same author (8) investigated the latent period in the action of CuCl₂ on respiration in *Aspergillus niger*. There is first a period during which there is no change in the rate of the production of CO₂, following which the rate of respiration falls. This preliminary interval of no change is called the latent period.

Brooks (5) found that at low concentration of NaCl, KCl, CaCl₂, and MgCl₂ the rate of respiration of *Bacillus subtilis* remains fairly constant,

but at higher concentrations there is a gradual decrease in rate, and at certain other concentrations there is an increase. Mg of as high a concentration as $0.01M$ has little effect upon the rate of CO_2 production. At $0.03M$ there is an increase in the rate, while in high concentrations there is a gradual decrease.

Gustafson (12) states that in the presence of 0.05 percent dextrose the respiration of *Aspergillus niger* is increased by NaCl in concentration of $0.25M$ to $0.5M$ and by $0.5M$ $CaCl_2$. Stronger concentrations decrease the respiration.

Zaleski and Reinhard (37) show that KNO_3 , KH_2PO_4 , $NaNO_3$, NaH_2PO_4 , $MgSO_4$, $ZnSO_4$, at certain concentrations increase respiration of the seedlings of *Lupinus angustifolius*.

Montemartini (25) studied the effect of $MnSO_4$ and of $CuSO_4$ on the development, respiration, and photosynthesis of grapes, lupines, beans, potatoes, and flowers of *Leucanthemum* and of *Ageratum*. He compared the CO_2 liberated and the amount of the different substances absorbed by the plants in solution of Mn varying in concentration from 0.005 percent to 0.05 percent. Marked differences were noted in the sensitiveness of different plants as shown by respiration. Grapes are most sensitive, then beans, followed by potatoes. Flowers are more sensitive than leaves; unopened flowers more than those that have opened.

Copeland (9), experimenting with the metals Cu, Zn, Cd, Ag, and Hg on *Elodea*, *Callitriche*, a crucifer, fish, and frog larvae, states that the respiration may be stimulated by a small fraction of a lethal concentration. When the concentration is increased, the evolution of CO_2 becomes accelerated, sometimes reaching twenty-five times the normal.

Krzemieniewski (20), experimenting with the salts of Knopp's nutrient solution, discovered that the different salts have different effects on respiration. Potassium salts and the nitrates are the most active.

Ray (32) concludes that the effect produced by Fe shows a remarkable resemblance to that due to oxidizing enzymes. A 0.25 percent solution of chloroform causes an increase in the rate of CO_2 production in *Ulva* followed by a decrease; 0.5 percent solution shows a decrease only. When *Ulva* is killed and the oxidizing enzymes are destroyed, no CO_2 is produced unless H_2O_2 and $Fe_2(SO_4)_3$ are present. He also states that organic acids when treated with H_2O_2 and $Fe_2(SO_4)_3$ produce CO_2 at a rate that can be measured by the indicator method.

Lyons (23) believes that the phosphate ion acts as an accelerator of oxidizing enzymes, since it accelerates both aërobie and anaërobie respiration.

It is well established that anesthetics and many other organic compounds cause an increase in respiration, followed by a decrease. This effect is shown by Thomas (36), Laurens (21), and Irving (16) in their investigations on seedlings; and by Ewart (10) on *Elodea*.

Appleman (1) used ethyl bromid vapor on potatoes for one half hour and noted that the respiration was doubled.

Irwin and Weinstein (18) report that ethyl alcohol decreases the CO_2 formation in radish seedlings with the production of organic acids.

When fungi are investigated as to the effects of anesthetics and other organic compounds on respiration, the results are very similar to the preceding observations, as demonstrated by Brooks (3), Smith (34), and Gustafson (11).

MATERIALS AND METHODS

Lupinus albus was used throughout the experimental work reported here. Great care was taken in selecting uniform seeds in the hope of securing plants similar in size and vitality. The seeds were germinated in sand in small flower pots, from which it was very easy to remove them. After disposing of the adhering sand, the seedlings were transferred to water cultures. Every day a new lot of seeds in excess of the number used were planted in order to insure a regular large supply of plants of the same age, from which a selection of plants uniform in size and vigor could be made. The plants were ready for use at a uniform stage of development, as determined by their leaves. By this means variation in leaf surface was small. These plants so selected showed a vigorous growth in ordinary tap water for twelve to fourteen days. When ready for use they were transferred from the greenhouse to the constant temperature room in the evening. Here they remained in the dark during the following day while they were being used for experimental purposes. The temperature of this room was usually kept between 23°C . and 24°C ., and during any one experiment it never varied one half a degree. This temperature was usually within a few degrees of that of the greenhouses.

In order to measure the output of CO_2 , an apparatus similar to that designed by Osterhout¹ was used in connection with a color indicator. Since this apparatus is fully described elsewhere, only the modifications will be dealt with here.

The apparatus consists of a closed system in which there is a chamber to contain the plants, and a rotary blower so placed in the system as to draw air out of the plant chamber and force it through a tube containing the indicator, phenolsulphonphthalein. A small high speed motor controlled by a rheostat running on a steady current secures a uniform circulation of air from the plant chamber through the tube containing the indicator back to the plant chamber. The blower is about 8.5 cm. in diameter and 3.5 cm. in width.

The plant chamber was placed in a dark constant temperature room through the walls of which ran the glass tubes connecting it with the blower and indicator tube in an adjacent light room. The base of the plant chamber is formed by a pan containing mercury, which serves as a seal. Resting in this mercury is an inverted percolator jar, narrow, conical, and of a conveniently small size. This jar terminates in a small tube that

¹ Described in papers by Osterhout, Haas, Irwin, Cook, and others.

joins a short rubber tube connection, which is disconnected each time a new set of plants is placed in the chamber. The incoming tube rises from the bottom through the mercury and terminates in a bulb with lateral openings, which distributes the current of air entering the chamber. This dispersal and the narrow shape of the jar reduce the dead air spaces and the whole volume of air tends to move as the current of the system passes through the chamber and escapes through the outlet at its top.

The indicator solution is placed in a test tube 2×25 cm. fitted with a ground glass stopper, through which passes an intake tube bringing air from the plant chamber in the dark room. This tube terminates in a bulb with lateral openings and serves as a bubbler. The indicator solution contains 10 cc. of a 0.001 percent solution of the NaHCO_3 and four or five drops of a 2 percent solution of phenolsulphonphthalein. Four drops of indicator are usually sufficient to start, but if the shade of color does not match the color of the standard pH solution defined below, it is brought nearer the proper degree by running one or two drops of indicator down the side of the test tube so that droplets cling to the sides. These are added to the solution as desired by inclining the test tube. The solution is changed after each experiment, for constant bubbling evaporates the water, thus affecting the color and the end point; but the solutions are used several hours without much deterioration in the end point, or much variation in the reaction time, to be explained below.

The limits of the color change are fixed by two test tubes of buffer solutions made up of the phosphates according to Clark (6) and have a known pH standard of 7.73 and 7.38.

The light in which colors are compared comes from a one-hundred-watt daylight bulb, suspended at a certain distance above the indicator. This light must be constant and unvarying, otherwise there is interference with good matching of the colors.

A side branch, placed in the circuit between the plant chamber and the indicator tube, is connected to a large U-tube filled with NaOH that serves to remove the CO_2 from the enclosed air current. This NaOH tube collects moisture, which accumulates to such an extent that it is quite frequently necessary to remove the U-tube, drain and wash the contents, and refill.

The apparatus is made up largely of glass, but with a few rubber connections which offer flexibility and expedite the necessary connecting and disconnecting. Leakage through the rubber is prevented by a painting of shellac. It seemed to be wise to repeat this painting several times as there was cracking from time to time, especially where the rubber was handled. In order to be certain that the system is air-tight, it is imperative to test it occasionally. This is done by exhausting the CO_2 and running the apparatus for several hours. If there is no noticeable change in color during that time, the apparatus is sufficiently free from leakage and therefore no error from that source can enter the results.

It is absolutely necessary to maintain a constant temperature. By thermometer and thermograph it was possible to follow the temperature in the constant temperature room. In the room adjacent, in which most of the apparatus was set up, the temperature was read from a thermometer and regulated to agree with that in the constant temperature room. The temperature never varied more than $0.5^{\circ}\text{C}.$, which is sufficiently close.

The intensity of respiration was measured by the length of time required to change the indicator solution from an initial standard pH value of 7.73 to 7.38. A standard time was found by preliminary trials with the test plants placed in tap water and any departure from this standard time, following the transfer to salt solutions, indicated a change in CO_2 production due to the effect of the new medium. The time data indicating respiratory activity are therefore relative to a determined normal rate and do not represent absolute quantities.

The plants to be used were brought from the greenhouse into the constant temperature room the evening before the test, becoming adjusted during the night to the temperature and to darkness. By this means, photosynthesis could not interfere with the measurement of respiration. The food supply seemed to be sufficient to keep the plant in its usual, active state. In the plant chamber were placed a convenient number of uniform plants, usually two, three, or four, having a pair of well-formed leaves. The selection was determined by the general vigor of the plants and by the leaf surface. After some experience, it is quite easy to judge the amount of leaf surface required to give the color change in two to three minutes, a time most conducive to accuracy.

When starting with a new set of plants or a new indicator solution, outside air necessarily enters the system. In order to remove the CO_2 , the current is passed through the U-tube containing the NaOH for ten minutes, although less time often accomplishes this removal. The valve is then turned, throwing the plant chamber into the circuit, and the air then coming directly from the plants bubbles through the indicator solution, which becomes lighter in color due to the increasing H-ion concentration caused by the CO_2 from the plants dissolving in it. The rising CO_2 concentration induces a paling of the purple color of the indicator. A reading is made when this waning color matches a tube containing a known pH, which serves as the upper standard. On continuing, the pale pink of the lower known pH standard is reached and the time recorded. When the indicator solution is changed to match this lower standard, the air current is switched into the NaOH tube, which reduces the CO_2 concentration and this CO_2 -free air bubbling through the indicator removes the CO_2 dissolved in it, raises the pH, and restores the color.

It is absolutely necessary that the time of lowering the CO_2 concentration be constant throughout the experiment, for a shorter time results in a shorter reading. Two or three minutes effect an adequate reduction, for

it does not seem necessary to exhaust the enclosed air of CO_2 each time, as the principle of increasing and reducing the CO_2 concentration is involved. Time is wasted by carrying exhaustion far beyond the upper standard, for time will be needed for the return of the CO_2 concentration. Therefore, the time was calculated so that it required from one half to one minute to reach the upper standard after the current was switched from the NaOH tube.

Several readings were recorded until at least three were constant. They were taken as the normal rate of respiration. The plants thus calibrated were then changed from tap water to a salt solution, in which the stems were cut just above the ground line. By this means the solution enters the plant directly. After returning the plants into the chamber, the preceding operation was repeated. At regular intervals readings were recorded, giving points from which a curve representing the effect of the given solution could be drawn.

The chlorids of Cu, Cd, Al, Fe, Mg, and Na were used in these experiments. Various concentrations of each salt were tried in order to discover the ranges of molar concentration that cause a stimulation of the production of CO_2 . The range is practically of the same width for each metal but does not occur at the same molar concentrations for the different salts tried.

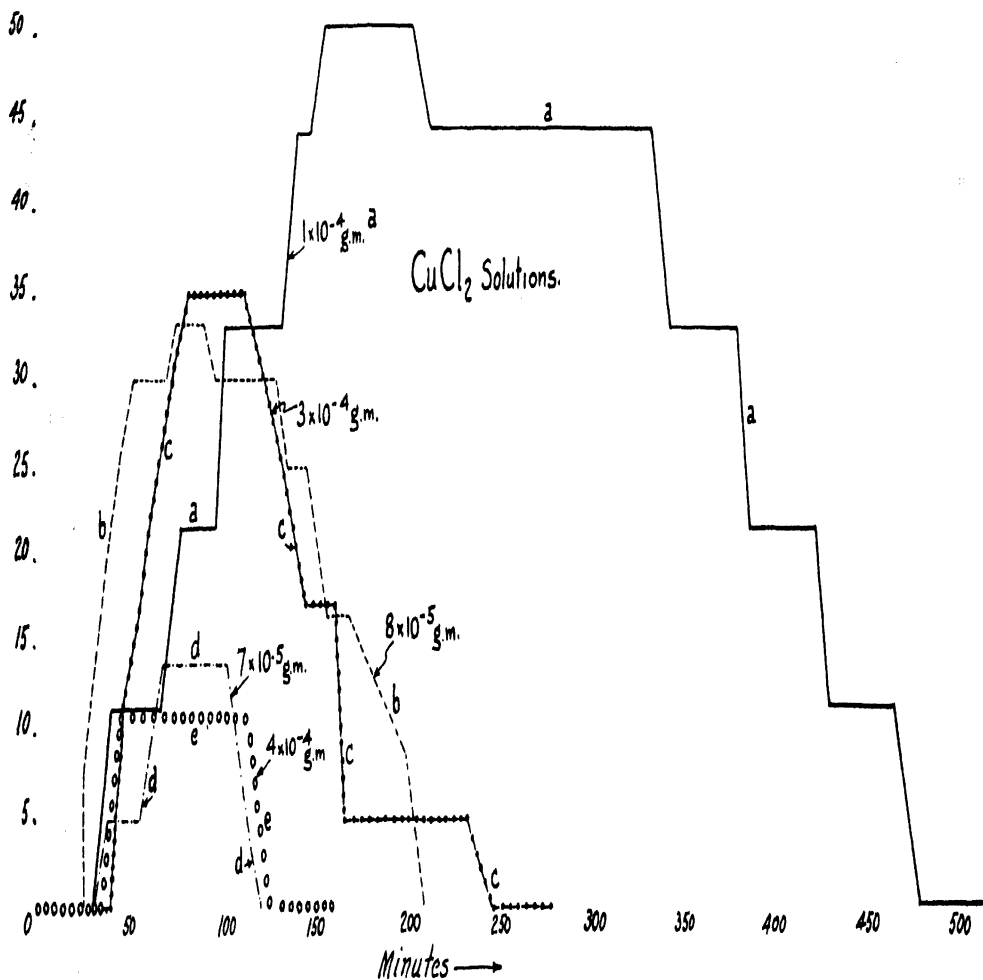
The reciprocal of the time required to change the color from pH 7.73 to 7.38 was taken as the rate of respiration or CO_2 production. After reaching the lower end point, the CO_2 was removed by passing the air through the NaOH after which the indicator returned to its original color. The time required to change it was again noted. By this means a series of readings was taken from which a curve was made by plotting time against the output of CO_2 . In general, they are of the same nature, but differ somewhat in length. Those within the range of stimulation exhibit an increase in CO_2 production, and then return to normal respiration, continuing so for several hours. Readings were not carried further after such a definite return was noted. Respiration rates in higher concentrations display a gradual decline from the normal rates. A plant cut off under distilled water showed no variation in respiration during a subsequent period of eight hours. The curves here shown are selected from a number of similar typical curves.

RESULTS

Copper Chlorid

The range of concentration causing a stimulation of CO_2 production extends from $4 \times 10^{-4}M$ to $7 \times 10^{-5}M$. Text figure 1 shows the manner of the rate of change in CO_2 production under the influence of concentrations of $1 \times 10^{-4}M$, $8 \times 10^{-5}M$, $3 \times 10^{-4}M$, $7 \times 10^{-5}M$, and $4 \times 10^{-4}M$ CuCl_2 .

Curve A represents a gradual increase in CO_2 production. After rising

% increase in CO_2 

TEXT FIG. 1. Respiration of *Lupinus albus* in solutions of $CuCl_2$. Curve *a* represents the respiration in $1 \times 10^{-4} M$; curve *b*, in $8 \times 10^{-5} M$; curve *c*, in $3 \times 10^{-4} M$; curve *d*, in $7 \times 10^{-5} M$; and curve *e*, in $4 \times 10^{-4} M$.

to fifty percent in one hundred and sixty minutes, followed by a slow descent, the normal rate of respiration returned following a lengthy stimulation of four hundred and seventy-eight minutes. This normal rate remained constant for two hours, after which time the readings were stopped.

In curve *B* the CO_2 output increased gradually during a period of eighty minutes to thirty-three percent above the normal rate and slowly descended to normal at the end of two hundred and ten minutes.

In curve *C* the increase in CO_2 was abrupt. When thirty-five percent was reached, at the end of eighty minutes, a rapid drop was seen which passed gently into the normal at the end of two hundred and forty-five minutes.

In curve *D* the increase in CO_2 of thirteen percent is small, due to a concentration low for stimulation. In curve *E* there is also a small increase of CO_2 , of eleven percent, small due to a concentration too high for maximal stimulation. The above curves are representative ones within the range of concentration causing stimulation of CO_2 production.

Table 1 shows this relation. Column *A* gives percentage of maximum increase in CO_2 production. Column *B* shows the time at the end of which stimulations begin, *i.e.*, the latent period, and *L* is the length of the stimulation period in minutes.

TABLE 1. *Stimulation of CO_2 Production in Copper Chlorid Solutions*

| Concentration of CuCl_2 Solutions | Percentage of Maximum Increase | Length of Latent Period | Duration of Stimulation |
|---|-----------------------------------|----------------------------|----------------------------|
| | <i>A</i> | <i>B</i> | <i>L</i> |
| $4 \times 10^{-4}M$ | 11 | 35 m. | 72 m. |
| $3 \times 10^{-4}M$ | 35 | 40 m. | 205 m. |
| $1 \times 10^{-4}M$ | 50 | 30 m. | 448 m. |
| $8 \times 10^{-5}M$ | 33 | 25 m. | 185 m. |
| $7 \times 10^{-5}M$ | 13 | 38 m. | 76 m. |

Concentrations of $5 \times 10^{-4}M$ and $6 \times 10^{-4}M$ CuCl_2 cause no increase in respiration; and after the duration of two hundred and ten minutes no decrease is evident. These solutions may therefore be regarded as lying outside the range of stimulating concentration.

In text figure 2 the curve represents the effect of a $0.001M$ solution of CuCl_2 on the production of CO_2 . There is a gentle decrease in respiration. Reduction starts at the end of about thirty-six minutes. In seventy-two minutes it reaches sixteen percent; in one hundred and eight minutes, forty-seven percent.

Cadmium Chlorid

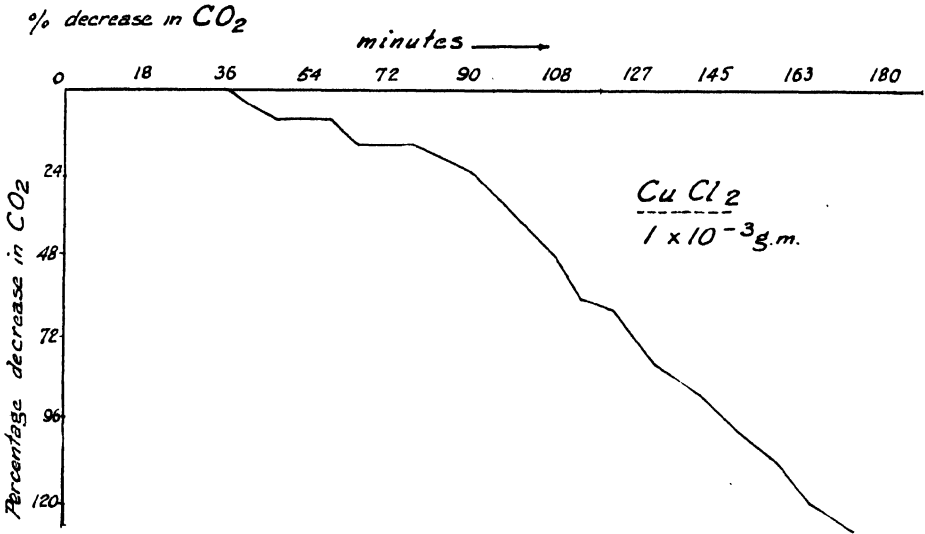
The range of stimulation of respiration for Cd occurs between 8×10^{-6} and 1×10^{-6} . Table 2 shows (*A*) the relation of the molar concentrations

to the percentage of increase, the beginning of stimulation (*B*), and the length of time the stimulation lasts (*L*).

TABLE 2. *Stimulation of CO₂ Production in Cadmium Chlorid Solutions*

| Concentration of Solutions | Percentage of Maximum Increase | Length of Latent Period | Duration of Stimulation |
|----------------------------|--------------------------------|-------------------------|-------------------------|
| | <i>A</i> | <i>B</i> | <i>L</i> |
| $8 \times 10^{-6} M$ | 30 | 33 m. | 79 m. |
| $6 \times 10^{-6} M$ | 40 | 10 m. | 158 m. |
| $4 \times 10^{-6} M$ | 22 | 47 m. | 137 m. |
| $3 \times 10^{-6} M$ | 18 | 39 m. | 110 m. |
| $2 \times 10^{-6} M$ | 16 | 70 m. | 49 m. |
| $1 \times 10^{-6} M$ | 5 | 69 m. | 38 m. |

The table shows that the stronger solutions cause a greater stimulation of CO₂ production. There is a correlation between the intensity of stimu-



TEXT FIG. 2. CO₂ production of *Lupinus albus* in 0.001 *M* CuCl₂.

lation and the length of the period, but a reverse order exists with reference to the beginning of stimulation.

Aluminum Chlorid

The range of stimulation of CO₂ production for Al exists between 3×10^{-5} and 6×10^{-6} . Stimulation is evident as early as twenty-two minutes after the salt enters the stem of the plant. Table 3 shows (*A*) the relation of the molar concentration to the percentage of increase, the time before stimulation begins (*B*), and the length of stimulation (*L*).

TABLE 3. *Stimulation of CO₂ Production in Solutions of Aluminum Chlorid (AlCl₃)*

| Concentration of Solutions | Percentage of Maximum Increase | Length of Latent Period | Duration of Stimulation |
|----------------------------|--------------------------------|-------------------------|-------------------------|
| | <i>A</i> | <i>B</i> | <i>L</i> |
| $3 \times 10^{-6}M$ | 8 | 60 m. | 31 m. |
| $2 \times 10^{-6}M$ | 17 | 22 m. | — |
| $1 \times 10^{-6}M$ | 21 | 26 m. | 71 m. |
| $9 \times 10^{-6}M$ | 44 | 22 m. | 110 m. |
| $8 \times 10^{-6}M$ | 20 | 48 m. | 79 m. |
| $7 \times 10^{-6}M$ | 13 | 43 m. | 73 m. |
| $6 \times 10^{-6}M$ | 7 | 65 m. | 16 m. |

If 9×10^{-6} is taken as the middle point, the percentage column *A* shows a decrease in both directions exactly at the same rate. In column *B* there is a tendency of the stronger solutions to act quickly. Column *L* shows a gradual decrease in either direction.

Sodium Chlorid

The range of stimulation of respiration for Na appears at the concentration of $4 \times 10^{-4}M$ to $7 \times 10^{-5}M$. The table below shows the relation of the molar concentrations to the percentage of increase, the time stimulation begins, and the length of stimulation.

TABLE 4. *Stimulation of CO₂ Production in Solutions of Sodium Chlorid (NaCl)*

| Concentration of Solutions | Percentage of Maximum Increase | Length of Latent Period | Duration of Stimulation |
|----------------------------|--------------------------------|-------------------------|-------------------------|
| | <i>A</i> | <i>B</i> | <i>L</i> |
| $4 \times 10^{-4}M$ | 11 | 60 m. | 5 m. |
| $3 \times 10^{-4}M$ | 11 | 60 m. | 14 m. |
| $2 \times 10^{-4}M$ | 24 | 50 m. | 41 m. |
| $1 \times 10^{-4}M$ | 26 | 48 m. | 50 m. |
| $9 \times 10^{-5}M$ | 20 | 45 m. | 29 m. |
| $8 \times 10^{-5}M$ | 11 | 44 m. | 14 m. |
| $7 \times 10^{-5}M$ | 7 | 60 m. | 10 m. |

The percentage of increase of respiration gradually decreases in both directions in the same manner from the high point at twenty-six percent. The extremes of the range are slower in beginning to act. The length of stimulation is quite brief, being somewhat shorter at either extreme of concentration.

Magnesium Chlorid

The range of stimulation of the production of CO₂ for Mg occurs from the concentrations of $5 \times 10^{-4}M$ to 8×10^{-5} . Table 5 shows the relation between the molar concentrations and the percentage of increase of respiration, the time after which stimulation begins, and the length of stimulation.

TABLE 5. *Stimulation of CO₂ Production in Solutions of Magnesium Chlorid (MgCl₂)*

| Concentration of Solutions | Percentage of Maximum Increase | Length of Latent Period | Duration of Stimulation |
|----------------------------|--------------------------------|-------------------------|-------------------------|
| | <i>A</i> | <i>B</i> | <i>L</i> |
| $5 \times 10^{-4}M$ | 9 | 45 m. | 10 m. |
| $3 \times 10^{-4}M$ | 19 | 23 m. | 68 m. |
| $1 \times 10^{-4}M$ | 30 | 17 m. | 70 m. |
| $8 \times 10^{-5}M$ | 9 | 56 m. | 26 m. |

The percentage of increase is less for the higher and lower concentrations. The beginning time is later for the same; while the length of stimulation is much shorter.

Ferric Nitrate

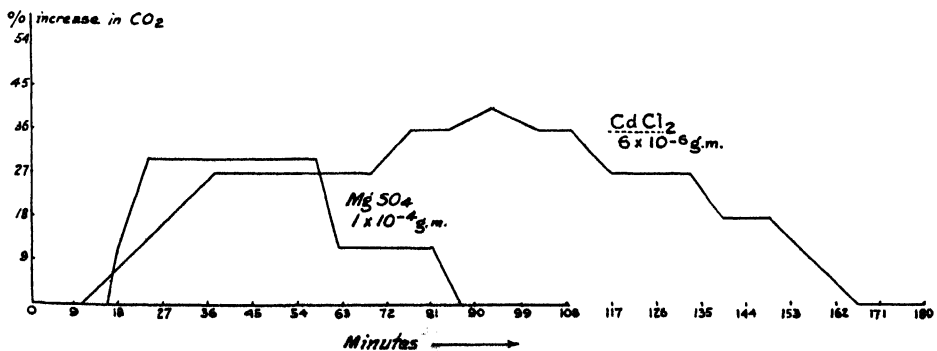
The range of stimulation of respiration of Fe is exhibited at the extremely low concentrations ranging from $9 \times 10^{-7}M$ to $4 \times 10^{-7}M$. Table 6 gives the relations of the molar concentrations to the percentage of increase of respiration, the time stimulation begins, and the length of stimulation.

TABLE 6. *Stimulation of CO₂ Production in Solutions of Ferric Nitrate Fe(NO₃)₃*

| Concentration of Solutions | Percentage of Maximum Increase | Length of Latent Period | Duration of Stimulation |
|----------------------------|--------------------------------|-------------------------|-------------------------|
| | <i>A</i> | <i>B</i> | <i>L</i> |
| $9 \times 10^{-7}M$ | 5 | 60 m. | 5 m. |
| $8 \times 10^{-7}M$ | 11 | 28 m. | 30 m. |
| $7 \times 10^{-7}M$ | 9 | 31 m. | 37 m. |
| $5 \times 10^{-7}M$ | 15 | 44 m. | 42 m. |
| $4 \times 10^{-7}M$ | 15 | 51 m. | 10 m. |
| $3 \times 10^{-7}M$ | 5 | 60 m. | 12 m. |

The table shows that the lower concentrations caused a greater increase in CO₂ production. The other two columns exhibit the usual regularity.

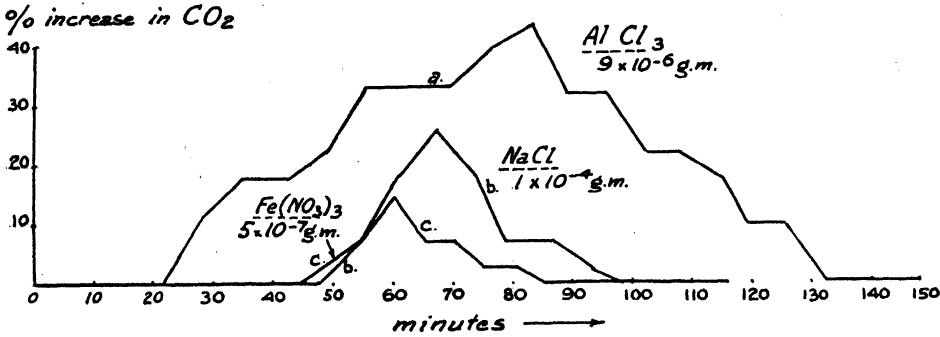
Text figure 3 shows the manner of the rate of change in CO₂ production under the influence of concentrations of $6 \times 10^{-6}M$ CdCl₂, and $1 \times 10^{-4}M$



TEXT FIG. 3. Curves showing the respiration of *Lupinus albus* at the maximum stimulation in solutions of CdCl₂ and of MgSO₄.

MgSO₄. These curves represent the highest percent of increase in CO₂ production seen in the compounds named. With MgSO₄ the CO₂ increases to thirty percent above normal rather rapidly, and returns to normal somewhat less abruptly, after seventy minutes. With CdCl₂ the CO₂ increases gradually to forty percent above normal, and returns at the end of one hundred and fifty-eight minutes.

Text figure 4 shows the manner of the rate of change in CO₂ production under the influence of a concentration of $9 \times 10^{-6}M$ AlCl₃, as represented by curve a, $1 \times 10^{-4}M$ NaCl, as represented by curve b, and $5 \times 10^{-7}M$ Fe(NO₃)₃, as represented by curve c. AlCl₃ produces an increase in CO₂



TEXT FIG. 4. Curves showing the respiration of *Lupinus albus* at the maximum stimulation in solutions of AlCl₃ (curve a), NaCl (curve b) and Fe(NO₃)₃ (curve c).

which reaches forty-four percent and decreases to normal at the end of one hundred and ten minutes. NaCl exhibits a rapid rise in CO₂ to twenty-six percent and in the same degree returns to normal at the end of fifty minutes. Fe(NO₃)₃ shows the small increase of fifteen percent, which endured just forty-two minutes.

DISCUSSION

It appears clear from the above evidence that for the salts of the metals here studied a definite and characteristic range of dilution exists which produces stimulation of CO₂ production in *Lupinus* seedlings. In table 7 are shown the maximum and minimum concentrations producing such stimulations in the case of the salts here dealt with.

TABLE 7. Concentration Range of Solutions Causing Stimulation to Respiration in *Lupinus albus*

| Salts | Stimulating Maximum | | Concentration Minimum |
|---|---------------------|----|-----------------------|
| MgSO ₄ | $5 \times 10^{-4}M$ | to | $8 \times 10^{-5}M$ |
| CuCl ₂ | $4 \times 10^{-4}M$ | " | $7 \times 10^{-5}M$ |
| NaCl..... | $4 \times 10^{-4}M$ | " | $7 \times 10^{-5}M$ |
| AlCl ₃ | $3 \times 10^{-5}M$ | " | $6 \times 10^{-6}M$ |
| CdCl ₂ | $8 \times 10^{-6}M$ | " | $1 \times 10^{-6}M$ |
| Fe(NO ₃) ₃ | $9 \times 10^{-7}M$ | " | $4 \times 10^{-7}M$ |

Mg, Cu, and Na are found practically in the same group, which is the least effective one so far as causing stimulation is concerned. The concentrations of Al and Cd producing stimulation are lower, but Fe reaches an extreme dilution, with a marked gap between itself and Cd. This seems to show that Fe stands out in some way from the other metals in its effect on respiration. Respiration does not seem to benefit by more Fe. All Fe solutions are toxic except in very great dilution, and then the length of stimulation is short and the percentage of increased CO₂ production is small.

The H-ion concentration of the salt solutions here used was determined previous to contact with the seedlings and the values obtained give the following kation series: Na < Mg < Cd < Cu < Al < Fe. In this arrangement there seems to be no relation between the pH of the salts and their effect on stimulation. Mg and Cd are closely associated in pH value, but the difference of their effect here is quite marked. Also the distinctly reverse order of Al and Cd should be noted.

The same arrangement of the kations is exhibited when the greatest percentage of CO₂ production is compared with the greatest length of stimulation period, as shown by table 8.

TABLE 8. *Degree and Duration of Stimulation of Respiration in Solutions Giving Maximal Values*

| Salts | Maximum Stimulation | Maximal Stimulation Period |
|---|---------------------|----------------------------|
| CuCl ₂ | 50% | 448 m. |
| AlCl ₃ | 44% | 110 m. |
| CdCl ₂ | 40% | 158 m. |
| MgSO ₄ | 30% | 70 m. |
| NaCl..... | 26% | 50 m. |
| Fe(NO ₃) ₃ | 15% | 42 m. |

This grouping does not coincide with the pH order nor with that of molar concentration. Here Fe and Na are associated, but above they occupy extremes in both cases.

All of the salts employed in these experiments are considered more or less toxic to plants, but the degree of toxicity is not correlated with any of the discovered results relating to respiration. According to True (unpublished paper), the kations here concerned have the following toxic equivalents: Na, 16; Mg, 750; Fe, 25,600; Cu, 25,600; Cd, 138,888. The toxic equivalent as here used is the maximal concentration permitting a trace of growth in *Lupinus* roots during the first 24-hour period following their transfer to the salt solutions in question. This is stated in the number of liters in which a gram equivalent of the salt is dissolved.

Considering the range of stimulation, the solutions of Na, Mg, and Cu are of about the same concentration, while their toxicity indicates a wide difference. Cd and Fe stand in a distinctly reverse order, and Na is

strikingly effective in its action on respiration. Cu gives 50 percent increased CO_2 production and Fe 15 percent, yet their toxic equivalents are approximately alike.

CONCLUSIONS

1. A stimulation of CO_2 production occurs in seedlings of *Lupinus albus* when placed in solutions of CuCl_2 , CdCl_2 , AlCl_3 , NaCl , MgSO_4 , and $\text{Fe}(\text{NO}_3)_3$.

2. The ranges of concentrations that stimulate CO_2 production are 5×10^{-4} to $8 \times 10^{-5} M$ MgSO_4 ; 4×10^{-4} to $7 \times 10^{-5} M$ CuCl_2 ; 4×10^{-4} to $7 \times 10^{-5} M$ NaCl ; 3×10^{-5} to $6 \times 10^{-6} M$ AlCl_3 ; 8×10^{-6} to $1 \times 10^{-6} M$ CdCl_2 ; and 9×10^{-7} to $4 \times 10^{-7} M$ $\text{Fe}(\text{NO}_3)_3$.

3. The highest percentages of increase of CO_2 production are: Cu, 50; Al, 44; Cd, 40; Mg, 30; Na, 26; Fe, 15.

4. The percent of increase of respiration for each metal is not correlated with its degree of toxicity.

5. The maximal period of stimulation of respiration is: Cu, 448 minutes; Cd, 158 minutes; Al, 110 minutes; Mg, 70 minutes; Na, 50 minutes; and Fe, 42 minutes.

The writer wishes to acknowledge his indebtedness to Professor Rodney H. True for the suggestion of the problem and for valuable help and criticism, to Professor William Seifriz he wishes to express his sincere appreciation of the interest and the generous assistance given throughout the experiments.

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GROWTH AND NITROGEN METABOLISM OF SQUASH SEEDLINGS.¹ II. WITH RESPECT TO STAGES OF DEVELOPMENT AND THE INFLUENCE OF LIGHT

MARY E. REID

(Received for publication November 30, 1929)

In a previous publication (6) it was shown that the leaves of squash seedlings having available a limited supply of nitrogen turn yellow at a relatively early age and that the rate at which yellowing occurs varies with the season of the year in which the seedlings are grown. The process occurs much more rapidly during the long-day months when the light intensity is high. This paper gives the results of a more detailed study of the changes in the leaves and other organs of the seedlings during progressive stages of development. The quickness with which the changes develop in plants which have a limited nitrogen supply and which synthesize carbohydrates rapidly has facilitated such an investigation.

Some study has been made of the changes in total nitrogen and protein content of leaves at different stages of development in normally grown mature plants. Mothes (5) found that after reaching a maximum content of nitrogen, mature leaves exhibit a continuous outward migration of nitrogen at the expense of protein and that hydrolysis of protein proceeds independently of the carbohydrate content. It appears that ageing leaves gradually lose the capacity to regenerate proteins from their split products. Even in the presence of an abundance of carbohydrates, protein decomposition occurs which eventually leads to yellowing and finally to the death of the leaves. The minimum protein content is found at the stage of yellowing. In narcosis experiments, conducted with detached leaves of *Phaseolus*, Mothes observed that protein is broken down in young leaves only when there is lack of carbohydrates. Detached young leaves effect a synthesis of protein at night, old leaves a decomposition of protein. Young leaves in spite of a higher total nitrogen content take up more nitrogen than old leaves when placed in a solution containing 0.5 percent asparagin and 5 percent glucose. The young leaves under these conditions also synthesize more protein than old leaves.

Smirnow (9) studied the carbohydrate and nitrogen content of sunflower and tobacco leaves at different stages of development. He observed that in sunflower leaves there is a depression in total carbohydrates and in protein nitrogen per unit of leaf surface at the time of flowering. The

¹ These investigations were conducted during 1926-8 in the Department of Physiological Chemistry of Yale University where the writer held a Sterling Research Fellowship.

decrease of total nitrogen during blooming is caused by its diversion to the flowering parts and to the developing upper leaves. There is apparently a decreased synthesis of protein at this time and a decrease in the reserves of poly- and disaccharids. However, the active forms of the carbohydrates, the monoses, exhibit a sharp increase. He considers that at this time in the life of the plant the synthesis of protein in the leaves is apparently limited through the same factors as toward the end of their life, in the period of yellowing. There is a decrease of respiratory energy, in spite of the higher monose content. The same facts are to be observed in the results obtained with tobacco leaves. At the end of flowering of sunflower plants, there is an increase of total nitrogen and protein nitrogen per unit of leaf surface and an increase in carbohydrates at the same time, although the monoses decrease. The distribution of nitrogen compounds in leaves of the same age of fruiting and topped plants is very characteristic. In the latter there is a greater content of total nitrogen and total carbohydrates, especially monoses, but the quantity of protein nitrogen is not greater than in normal fruit-bearing plants. The decrease in translocation from the leaves of topped plants which occurs with an accumulation of monoses and non-protein nitrogen brings about no increase in protein nitrogen. He did not find a lessened capacity for protein synthesis in ageing leaves as had Mothes (5) for legumes. It is possible that a decrease in synthesizing capacity occurs at a later stage but the late stages of sunflower leaves were not studied.

In tobacco leaves the total nitrogen and protein nitrogen do not accumulate after the blooming period as they do in leaves of sunflower. The relative content of protein nitrogen in percent of total nitrogen reaches its maximum in young leaves before blooming and decreases continuously to yellowing. The content of monoses per unit of leaf surface is lowest in the period previous to blooming and highest during the blooming period. After blooming the monose content decreases but increases again during the period of yellowing. The topping of tobacco plants before the formation of flower buds retards the decrease of total and protein nitrogen per unit of leaf surface in spite of the fact that growth in the topped plants is increased. The increase of protein in the topped plants at the adult stage of the leaf coincides with an increase of respiratory energy although at this time the leaves contained a smaller quantity of monoses. The results led the author to the conclusion that the content of protein at different stages in the life of a leaf is determined not only by the plastic materials necessary for the synthesis of protein but also by the quantity of energy available for respiration.

The observation may also be made from Smirnow's results that at those stages in the life of a leaf in which the protein content is high, the monose content is low, and those in which the protein content is low, the monose content is high. It seems that this inverse relation of monoses and leaf proteins may not be without significance.

Combes (1) followed the migration of nitrogen from the leaves into the shoots and roots of young beech and oak trees during the course of autumnal yellowing. His results are of special value because his data permitted the calculation of the absolute amounts of nitrogen in the roots, stems, and leaves at different periods during the latter part of the summer and autumn and the balance of nitrogen in the plant was thereby established. His results show that in yellowing, leaves lose about half of their nitrogen and the portion which is lost accumulates first in the stem, but later some goes into the roots. The migration of nitrogen from the leaves to the shoots goes on during the entire period of yellowing, from the beginning of September to the end of October, but it is accelerated just at the death of the leaf. During the month of August the content of nitrogen in the different organs did not vary, although at this time the leaves were less green than they had been during the latter part of July. The diminution of green pigments thus begins in summer, rather than abruptly in autumn. However, it appears that the migration of nitrogen from the leaves to the shoots does not begin until September when yellow patches develop, resulting from the local disappearance of all of the chlorophyll.

Riszmüller (7) determined the protein content of beech leaves at different times of year and found that it increases from May to July and diminishes from July to November.

Dulk (2) also investigated the protein content of beech leaves at different periods. He observed an increase from June to August and a decrease from August to November.

The decrease in chlorophyll observed by Combes in beech leaves during the month of August is doubtless to be connected with the diminution of protein content found by Riszmüller and Dulk. This break-down of protein thus probably occurs near or at the time of a decrease in chlorophyll but somewhat in advance of the migration of nitrogen from the leaf.

That the nitrogen lost by leaves before their fall originates wholly or partially from protein is shown by results of determinations of Schulze and Schütz (8) on leaves of *Acer Negundo*. In 200 leaves of about the same size they found:

| | |
|----------------|--------------------|
| May 7..... | 0.64 gm. protein N |
| June 6..... | 0.96 " " " |
| July 5..... | 1.20 " " " |
| Aug. 2..... | 0.78 " " " |
| Sept. 3-6..... | 0.81 " " " |
| Sept. 25..... | 0.50 " " " |

METHODS

The squash seeds were placed in germinators June 1 and planted in sand June 8. The seedlings were pushing through the surface of the sand on June 10 when the first nutrient solutions were applied. On June 18

and each day thereafter, 200 cc. of the nutrient solution were given to the cultures receiving ammonium nitrate, as at this time the plants had become so large that the 100 cc. amount was insufficient to maintain growth at a rapid rate. The changes in nitrogen content of leaves during growth, maturity, and decline were followed closely in the plants grown without an external nitrogen supply. Daily observations of growth and changes in the color of the leaves were made. Frequent observations were also made of the appearance of the chloroplasts and of the starch content by examining sections of the leaves. The Flückiger reaction² was used to detect quantitative differences in the content of free-reducing substances at different periods of development of the leaves. Leaves from different nodes obtained from plants harvested at different stages of development were dried and analyzed separately. Harvests of plants were made on June 15, 19, 25, 29, and July 6. The calculations in tables 1-4 are based on the quantitative results obtained with thirty-four seedlings.

RESULTS

Seedlings Grown in Normal Length of Day, in Normal Daylight, and in Nutrient Medium Lacking Nitrogen

The first harvest was made on June 15, one week after the seedlings were planted in the sand. During every day of this period there was a high light intensity. The seedlings grew very rapidly. The Kjeldahl analyses showed that about half of the nitrogen stored in the cotyledons had been mobilized and translocated (table 1). The first leaf of most of the seedlings had expanded, but a few were still folded along their mid-ribs. The rapidly growing roots contained about half of the nitrogen that had moved out of the cotyledons. A high nitrogen content and rapid early growth of roots had previously been observed in seedlings grown in May (6).

Another lot of seedlings was harvested on June 19 after they had grown in the sand and nutrient medium for 11 days. There was continuous sunshine during the days from June 15 to 18 inclusive. June 19 was a cloudy day. At this time the cotyledons had become yellowish-green in color. The many hours of sunshine, the high intensity of the light, and the lack of an external supply of nitrogen had apparently been responsible for the decided change in color within so short a period. The cotyledons had not grown since the previous harvest and they contained slightly more than one-third of their original store of nitrogen. They contained large amounts of starch and free-reducing substances. The first leaves also exhibited evidences of color change. Near the margins especially there

² A few crystals of copper tartrate were dissolved in a drop of 20 percent NaOH on a slide. Sections of the fresh tissues were placed in the solution and allowed to stand in a warm place (45° C.) for 20 minutes, after which they were examined microscopically for the amount of copper oxid crystals.

TABLE I. *Seedlings Grown in Solution Lacking Nitrogen and Harvested at Different Stages of Growth. Thirty-four Plants. Seeds Contained 328 mg. N. June 8-July 6, 1928*

| Age of Seedlings | Organs of Seedlings | Green Weights (gm.) | Dry Weights (gm.) | Total Nitrogen (mg.) | Percentage of Nitrogen in Dry Weight | Percentage of Nitrogen in Green Weight | Percentage of Moisture |
|--|---------------------|---------------------|-------------------|----------------------|--------------------------------------|--|------------------------|
| Normal-length-of-day seedlings grown in open in greenhouse | | | | | | | |
| June 15 Seedlings grown in sand seven days | Leaf I | 6.49 | 0.690 | 40 | 5.80 | 0.616 | 89.4 |
| | Stems + petioles | 20.09 | 1.428 | 34 | 2.41 | 0.171 | 92.8 |
| | Roots | 43.50 | 2.210 | 78 | 3.52 | 0.179 | 94.9 |
| | Cotyledons | 50.02 | 4.488 | 152 | 3.38 | 0.303 | 91.0 |
| | Total | 120.10 | 8.816 | 304 | | | |
| | Loss of N | | | -24 | | | |
| June 19 Seedlings grown in sand eleven days | Leaf I | 10.77 | 1.598 | 56 | 3.49 | 0.521 | 85.1 |
| | Leaf II (buds) | 0.68 | 0.126 | 7 | 5.63 | 1.050 | 81.5 |
| | Stems + petioles | 22.98 | 2.329 | 39 | 1.70 | 0.171 | 89.8 |
| | Roots | 60.86 | 2.499 | 75 | 3.02 | 0.124 | 95.9 |
| | Cotyledons | 48.96 | 4.386 | 114 | 2.61 | 0.234 | 91.0 |
| | Total | 144.25 | 10.938 | 291 | | | |
| | Loss of N | | | -37 | | | |
| June 25 Seedlings grown in sand seven- teen days | Leaf I | 12.75 | 2.030 | 53 | 2.62 | 0.417 | 84.1 |
| | Leaf II | 5.78 | 0.809 | 28 | 3.52 | 0.492 | 86.0 |
| | Leaf III (buds) | 1.02 | 0.136 | 7 | 5.43 | 0.733 | 86.6 |
| | Stems + petioles | 35.02 | 4.012 | 60 | 1.50 | 0.172 | 88.5 |
| | Roots | 71.06 | 2.890 | 77 | 2.65 | 0.107 | 95.9 |
| | Cotyledons | 51.00 | 4.352 | 71 | 1.62 | 0.138 | 91.4 |
| | Total | 176.63 | 14.229 | 296 | | | |
| | Loss of N | | | -32 | | | |

TABLE I.—*Continued*

| Age of Seedlings | Organs of Seedlings | Green Weights (gm.) | Dry Weights (gm.) | Total Nitrogen (mg.) | Percentage of Nitrogen in Dry Weight | Percentage of Nitrogen in Green Weight | Percentage of Moisture |
|--|---------------------|---------------------|-------------------|----------------------|--------------------------------------|--|------------------------|
| June 29 Seedlings grown in sand twenty- one days | Leaf I | 10.71 | 1.717 | 38 | 2.19 | 0.351 | 83.8 |
| | Leaf II | 8.30 | 1.290 | 34 | 2.65 | 0.411 | 82.0 |
| | Leaf III | 3.31 | 0.523 | 20 | 3.82 | 0.606 | 84.2 |
| | Leaf IV | 1.03 | 0.153 | 7 | 4.35 | 0.646 | 85.2 |
| | Leaf V | 0.34 | 0.0156 | 1 | 6.41 | 0.294 | 95.4 |
| | Stems + petioles | 38.42 | 4.962 | 52 | 1.05 | 0.135 | 87.1 |
| | Roots | 80.24 | 4.318 | 78 | 1.80 | 0.097 | 94.6 |
| | Cotyledons | 41.48 | 3.434 | 42 | 1.17 | 0.101 | 91.6 |
| | Total | 183.83 | 16.413 | 272 | | | |
| | Loss of N | | | -56 | | | |
| July 6 Seedlings grown in sand twenty- eight days | Leaf I | 11.90 | 1.363 | 23 | 1.67 | 0.191 | 88.5 |
| | Leaf II | 10.57 | 1.374 | 28 | 2.04 | 0.265 | 87.0 |
| | Leaf III | 6.80 | 0.996 | 25 | 2.53 | 0.370 | 85.3 |
| | Leaf IV | 3.40 | 0.510 | 15 | 2.91 | 0.437 | 85.0 |
| | Leaf V | 1.27 | 0.063 | 3 | 4.06 | 0.204 | 95.0 |
| | Stems + petioles | 45.73 | 5.780 | 63 | 1.09 | 0.138 | 87.3 |
| | Roots | 88.74 | 4.080 | 80 | 1.95 | 0.089 | 95.4 |
| | Cotyledons | 15.64 | 2.958 | 36 | 1.22 | 0.231 | 81.1 |
| | Total | 184.05 | 17.124 | 273 | | | |
| | Loss of N | | | -55 | | | |

appeared to be considerable yellow blended with the green. Their average length at this time was 2.8 cm. and their width at the broadest part was 3.3 cm. The average area per leaf was 7.36 sq. cm. Six of the 37 seedlings harvested at this time had twin leaves at the first node, *i.e.*, the two leaves developed simultaneously. The plants having twin leaves at the first node had smaller leaves at the second node than plants with the normal leaf arrangement. It was decided that age should be the criterion for classification and both of the twin leaves were included with the leaves from the first node. Consequently, in this set of seedlings the leaf area and nitrogen content of leaf I are slightly larger and those of leaf II slightly smaller than if there had been the normal leaf arrangement. The first leaves at this time contained very large amounts of starch and moderate amounts of reducing substances. The roots grew rapidly during the period from June 15 to 19 but there was no change in their total amount of nitrogen. The stems did not gain noticeably in length but there was a slight increase in the amount of nitrogen contained in them. The greatest change in the stems was a large gain in dry weight, due chiefly to an accumulation of starch (table 1).

Another harvest was made on June 25. Between June 19 and 24, inclusive, there were only two hours of sunshine. During the interval of cloudy weather the seedlings developed characteristics such as had previously been noted in plants grown during December (6). The leaves recovered from the evidences of yellowing and became decidedly green with no trace of yellow apparent. They also increased markedly in size. The average area changed from 7.36 sq. cm. to 14.11 sq. cm. Only two of the 20 plants harvested had twin leaves at the first node. The leaves from the second node had developed rapidly and had an average area of 6.93 sq. cm. per leaf. Not many of the third set of leaves were expanded. The data presented in column 5 of table 1 show that the leaves from the first node harvested on June 19 contained 56 mg. of nitrogen and the corresponding leaves of June 25 contained 53 mg. If there had not been so many twin leaves in the harvest on June 19, the relative amounts of nitrogen found in the leaves at the two harvests might possibly have been reversed. Apparently the change in the leaves at the first node between June 19 and 25 was chiefly in cell expansion and possibly with the expansion there was a flattening out of the leaf in consequence of the low light intensity. There must have been some change in the form of some of the nitrogenous constituents, however, associated with the apparent change in color. It is supposed that a restoration of the chloroplasts from the decomposition effects previously observed had occurred. On June 25 there was no evidence of degeneration of the chloroplasts. They appeared to be in as green and healthy a condition as those of the second set of leaves. Between June 19 and 25 the nitrogen contained in the leaves from all the nodes increased from 63 to 88 mg. The cotyledons were somewhat yellower than on June

19 and their nitrogen diminished from 114 to 71 mg. Although the roots had gained in weight, the amount of nitrogen found in them had not changed. The green weights of the stems increased from 23 to 35 g. and their content of nitrogen from 39 to 60 mg.

From June 25 to 29 there was sunshine most of the daytime. On June 29, the cotyledons had scarcely any green color left in them. Eleven of the 41 plants harvested had the cotyledons partially withered as a result of exhaustion of the reserves. During the four-day period since the previous harvest, their dry weight as well as their green weight and the total amount of nitrogen within them diminished. During the four-day period in which the days were all sunny, all organs of the plant except the cotyledons and leaves from the first nodes increased both in green and dry weight. The plants lost some of their former greenness. A decrease in the starch content was observed but microchemical tests indicated that reducing substances were present in larger amounts than in the earlier stages. The total amount of nitrogen found in the first leaves decreased from 53 to 38 mg. and the nitrogen per square centimeter of leaf surface diminished from 0.111 mg. to 0.078 mg. (table 4, column 5). The leaves from the second node gained in average area from 6.9 to 9.9 sq. cm. The nitrogen found in them increased from 28 to 34 mg. but the amount of nitrogen per square centimeter of leaf surface decreased from 0.121 mg. to 0.101 mg. Most of the gain in leaf area and content of nitrogen was made by the leaves at the third and fourth nodes. The total amount of nitrogen contained in all the leaves increased from 88 to only 100 mg. since June 25, whereas 29 mg. of nitrogen were translocated from the cotyledons during this period. The second to fifth leaves, inclusive, gained 27 mg. of nitrogen since the last harvest and the first set of leaves lost 15 mg. The 27 mg. of nitrogen gained by the growing leaves may have come exclusively from the cotyledons or partially from the nitrogen lost from the oldest set of leaves or from the stems. Notwithstanding the fact that the first set of leaves had lost 15 mg. of nitrogen since the last harvest, it appeared that the stems had also lost some of the nitrogen that was found in them on June 25. The stems grew longer during this period but gained in dry weight relatively more than in green weight. The roots gained in number, length, and in green and dry weights but not in their holding of nitrogen. There appeared to be unexplainable losses of nitrogen from the seedlings during this period. Smaller losses had also been found at the previous harvests.

There was sunshine during most of the day time between June 29 and July 6. An unutilizable residue of 36 mg. of nitrogen was found in the cotyledons on July 6. The yellowing and drying of the cotyledons had advanced very rapidly since the previous harvest. All of them were withered. Their starch and water content had diminished markedly. The total fresh and dry weights of the leaves increased but the amount of chlorophyll appeared to have greatly diminished. Even the younger

leaves had a yellowish-green color. Some of the first set of leaves had begun to wither. The chloroplasts of the palisade tissue had degenerated; only fragments of them were to be observed. The nitrogen found in the first or oldest set of leaves was 23 mg., less than half the amount found in them on June 19 and 25. The nitrogen per square centimeter of leaf surface diminished from 0.078 to 0.048 mg. since the previous harvest (table 4). The chlorophyll and plasma content of the chloroplasts of the second leaves appeared to be much less than at the previous harvest. The nitrogen contained in them diminished from 34 mg. to 28 mg., a loss of only 6 mg. The results indicate that loss of green color and plasma from the chloroplasts precedes by an interval the decline in nitrogen content of the leaves. If all of the resulting products of chloroplast decomposition move out of the leaf into the axial organs, the translocation does not happen so rapidly as the break-down processes occur. The third leaves were still growing at the time the harvest was made. Their green and dry weights had approximately doubled since the previous harvest. Their total nitrogen, however, increased from only 20 mg. to 25 mg. The fourth and fifth leaves were growing and gaining in nitrogen. The total nitrogen contained in the total number of leaves decreased between June 29 and July 6 from 100 to 94 mg. The amount of nitrogen in the third, fourth, and fifth leaves increased in amount by 15 mg. whereas the first and second leaves lost 21 mg. and the cotyledons about 6 mg. It might be concluded that some of the nitrogen liberated from the first and second set of leaves is reutilized in the growth of the younger leaves. This possibly did occur to a small extent.

The stems grew longer during the period from June 29 to July 6 and the nitrogen found in them increased from 52 to 63 mg. The roots also grew somewhat longer but there was not a significant gain in the amount of nitrogen found in them.

The total green weight of the plants did not increase during this last period but the dry weights gained slightly. The percentage of nitrogen in the dry weight decreased in all of the leaves but in none of the other organs. Figure 5 of Plate XXIX shows one of the seedlings harvested on July 6. Unfortunately the third leaf was not adjusted so as to permit a view of other younger leaves at the apex of the stem.

The Balance of Nitrogen in the Seedlings

Because of unexplainable losses of nitrogen from seedlings grown during May and June of the previous year the following possibilities as sources of loss were considered in this experiment.

I. Loss during drying in vacuum oven. Bottles containing sulfuric acid were connected with the exit from the vacuum oven in such a way that the air coming from the drying chamber was drawn through the acid so as to absorb possible traces of ammonia or any basic form of nitrogen that

could be absorbed by the acid. A Kjeldahl analysis of the acid was made. It was found that no basic nitrogen had been given off during drying by any of the five lots of material.

II. Losses due to leaching of nitrogen from the roots into the sand. An attempt was made to answer this question by saving the daily washings from the sand. These solutions were acidified with sulfuric acid and collected in a large bottle kept tightly stoppered. Aliquots of the solution were evaporated just to dryness and Kjeldahl analyses were made. A loss of not more than 5 mg. of nitrogen was found for the 34 seedlings indicated in the calculations in table I.

III. Losses in washing roots with water. The water used in washing the roots was acidified and evaporated just to dryness. A nitrogen determination of the residue indicated a loss of 1.4 mg. from 34 seedlings.

IV. Losses due to material clinging to watch glasses in which tissues were dried. The Kjeldahl determination showed a loss of 1.22 mg. of nitrogen. Total losses of nitrogen so far as could be determined were:

| | |
|---|----------------|
| Loss in drying | 0.0 mg. |
| Water soluble nitrogen lost in sand | 5.0 mg. |
| Losses in washing roots | 1.4 mg. |
| Losses on drying dishes | 1.22 mg. |
| Total | <hr/> 7.62 mg. |

Undoubtedly there were traces of nitrogen left in the sand which were not removed by washing. It is supposed that losses might occur from sloughing off of cells from the root caps and from injury to the root hairs in removing the plants from the sand. The action of microorganisms in absorbing and possibly in breaking down nitrogenous compounds excreted by the roots is also considered as a possible source of loss of nitrogen.

Combes (1) also found unexplainable losses of nitrogen from two-year-old oak and beech trees during September. The decrease in September was followed by an increase during October. The loss of nitrogen in September is correlated with a diminution of about the same magnitude in the nitrogen content of the roots. He suggests that at this time a part of the nitrogen may be excreted into the soil and later may be re-absorbed. He states that in order to answer the question definitely, nitrogen determinations should be made of the nutrient medium in which the plants are grown as well as of the plants themselves.

It is possible that discrepancies in the nitrogen balance may result from a failure of the Kjeldahl method to recover a certain form or forms of nitrogen produced under conditions for growth such as were used in these experiments. The results of the analyses should indicate more of an accumulation of nitrogen in the stems during the later stages of development if the nitrogen lost from the leaves in consequence of the degenerative changes accumulates and remains chiefly in the stems.

TABLE 2. *Seedlings Grown in Solution Containing Nitrogen. Thirty-four Plants. Seeds Contained 324 mg. N. June 8-June 29, 1928*

| Organs of Seedlings | Green Weights (gm.) | No. of Times Green Weight is > than that of -N Seedlings of Same Age | Dry Weights (gm.) | Total Nitrogen (mg.) | Percentage of Nitrogen in Dry Weight | Percentage of Nitrogen in Green Weight | Percentage of Moisture |
|---|---------------------|--|-------------------|----------------------|--------------------------------------|--|------------------------|
| Seedlings grown in normal daylight and normal length of day | | | | | | | |
| Leaf I (oldest)..... | 53.4 | 5 | | | | | |
| Leaf II..... | 59.8 | 7 | | | | | |
| Leaf III..... | 57.8 | 17 | 21.84 | 1186 | 5.43 | 0.531 | 90.2 |
| Leaf IV..... | 38.4 | 37 | | | | | |
| Leaf V..... | 11.9 | 35 | | | | | |
| Leaf VI..... | 2.9 | — | | | | | |
| Stems and Petioles..... | 235.2 | 6 | 12.98 | 285 | 2.20 | 0.121 | 94.5 |
| Roots..... | 157.1 | 1.9 | 7.52 | 306 | 4.07 | 0.194 | 95.2 |
| Cotyledons..... | 62.2 | 1.5 | 3.06 | 70 | 2.30 | 0.113 | 95.1 |
| Total..... | 678.7 | | 45.40 | 1847 | | | |
| Gain in N..... | | | | +1523 | | | |

Seedlings Grown in Normal Length of Day, in Normal Daylight, and in Nutrient Medium Containing Nitrogen

The quantitative results are given in table 2 and the appearance of the plants is shown in figure 6, Plate XXIX. Only enough seedlings for one harvest could be grown. These were collected on June 29, when the seedlings had grown in the sand medium 21 days. Separate determinations of the nitrogen content of leaves from different nodes were not made. The carbohydrate content of the tissues was much lower than that of tissues of the seedlings grown without additional nitrogen. There was no definite evidence of degenerative changes in the leaves; even in the oldest leaves the chloroplasts were large, bright green in color, and had a fairly dense plasma content.

Seedlings Grown in Seven-hour Day, in Shaded Chamber, and Without an External Supply of Nitrogen

The seedlings were covered on all sides by a cheesecloth screen designed to reduce the light intensity. A cover of heavy black paper was drawn over the plants at 5 P.M. and removed at 10 A.M. the following morning. The quantitative results are given in table 3. Figure 3 illustrates the appearance of the plants.

The plants looked much like those previously grown during December (fig. 2), although the leaves were not quite so green. The blades were large, thin, flat rather than fluted at the margins as were those grown in full sunlight and they contained relatively large chloroplasts. The color was a fresh, although somewhat yellowish green like that of young leaves in the spring before they have fully developed their content of chlorophyll. The average areas per leaf (sq. cm.) from the first to the fifth node were as follows: 16.7, 15.5, 12.3, 9.5, and 3.0. The total leaf area per plant was 58 sq. cm., about the same as that of the December plants (60 sq. cm.). Areas of leaves from different nodes of plants grown in full sunlight and harvested on the same date (July 6) were 14.11, 10.26, 6.65, 3.96, and 0.85 sq. cm. respectively, making an average area of 36 sq. cm. per plant.

The entire set of leaves of the shaded plants contained 129 mg. of nitrogen on July 6; those of plants grown in the open contained 94 mg. The leaves of seedlings exposed to normal daylight contained when mature and before breakdown processes occurred considerably more nitrogen per unit area of leaf surface. The thinner leaves of the plants grown in the shaded chamber contained fewer cells and fewer chloroplasts per unit area of surface. It is evident from the data shown in table 4 that the older leaves of the shaded plants lost relatively much less of their nitrogen during the latter part of the experimental period than did those exposed to normal light. The first leaf of the plants grown in normal daylight used much more and the second leaf slightly more of the reserve nitrogen in its development than did the corresponding leaves of the shaded plants.

TABLE 3. *Table Showing Similarity in Growth and Allocation of Nitrogen between Seedlings Grown in June in Reduced Light and Seven-hour Days and Seedlings Grown in December under Normal Light Conditions. Thirty-four Plants. Seeds Contained 325 to 328 mg. of Nitrogen*

| Time of Harvest | Organs of Seedlings | Green Weights (gm.) | Dry Weights (gm.) | Total Nitrogen (mg.) | Percentage of Nitrogen in Dry Weight | Percentage of Nitrogen in Green Weight | Percentage of Moisture |
|--|---------------------|---------------------|-------------------|----------------------|--------------------------------------|--|------------------------|
| Seven-hour-day seedlings grown in shaded chamber and without additional nitrogen | | | | | | | |
| July 6 Seedlings grown in sand 28 days | Leaf I | 10.20 | 0.999 | 26 | 2.55 | 0.255 | 90.2 |
| | Leaf II | 8.50 | 1.003 | 30 | 2.98 | 0.352 | 88.2 |
| | Leaf III | 7.82 | 0.891 | 33 | 3.67 | 0.419 | 88.6 |
| | Leaf IV | 4.76 | 0.704 | 25 | 3.59 | 0.531 | 85.2 |
| | Leaves V & VI | 3.40 | 0.306 | 15 | 4.82 | 0.454 | 91.0 |
| | Stems + petioles | 60.86 | 5.746 | 68 | 1.18 | 0.111 | 90.5 |
| | Roots | 42.50 | 1.972 | 49 | 2.46 | 0.114 | 95.4 |
| | Cotyledons | 31.28 | 2.128 | 38 | 1.80 | 0.122 | 93.2 |
| | Total | 169.32 | 13.749 | 284 | | | |
| Seven-hour-day seedlings grown in shaded chamber and with additional nitrogen | | | | | | | |
| June 29 Seedlings grown in sand 21 days | Leaf I | 33.7 | 9.72 | 669 | 6.88 | 0.625 | 90.9 |
| | Leaf II | 32.3 | | | | | |
| | Leaf III | 27.2 | | | | | |
| | Leaf IV | 12.2 | | | | | |
| | Leaf V | 2.0 | 5.03 | 216 | 4.30 | 0.141 | 96.7 |
| | Stems + petioles | 153.0 | | | | | |
| | Roots | 47.6 | | | | | |
| | Cotyledons | 64.6 | | | | | |
| | Total | 372.6 | 19.76 | 1091 | | | |

TABLE 3.—*Continued*

| Time of Harvest | Organs of Seedlings | Green Weights (gm.) | Dry Weights (gm.) | Total Nitrogen (mg.) | Percentage of Nitrogen in Dry Weight | Percentage of Nitrogen in Green Weight | Percentage of Moisture |
|--|---------------------|---------------------|-------------------|----------------------|--------------------------------------|--|------------------------|
| Seedlings grown in November–December without additional nitrogen | | | | | | | |
| December 17 Seedlings grown in sand 32 days | Leaves | 31.40 | 4.040 | 147 | 3.64 | 0.469 | 87.1 |
| | Stems + petioles | 53.98 | 5.075 | 69 | 1.37 | 0.129 | 90.6 |
| | Roots | 28.01 | 1.775 | 51 | 2.85 | 0.180 | 93.7 |
| | Cotyledons | 35.99 | 2.342 | 51 | 2.17 | 0.140 | 93.5 |
| | Total | 149.38 | 13.232 | 318 | | | |
| Seedlings grown in November–December with additional nitrogen | | | | | | | |
| December 17 Seedlings grown in sand 32 days | Leaves | 122.2 | 13.24 | 723 | 5.49 | 0.593 | 89.2 |
| | Stems + petioles | 152.1 | 6.76 | 190 | 2.82 | 0.125 | 95.5 |
| | Roots | 57.7 | 2.72 | 126 | 4.70 | 0.221 | 95.5 |
| | Cotyledons | 67.6 | 3.09 | 102 | 3.28 | 0.150 | 95.4 |
| | Total | 399.6 | 25.81 | 1141 | | | |

The content of starch and free-reducing substances was much less than that found in leaves of seedlings grown in normal daylight. Degenerative changes in the leaves occurred more slowly and were less marked.

The green weight of the roots was greater than that of the plants which had been grown in December but the amount of nitrogen found in them was the same (table 3). The green weight of the roots was less than half that of the plants grown in normal daylight but the percentage of dry matter was the same. The stems were much like those of the December plants. They were more slender, taller, more succulent, contained smaller

TABLE 4. *Area and Nitrogen Content of Leaves of Seedlings of Different Ages Grown Without an External Supply of Nitrogen. Thirty-four Plants. Seeds Contained 328 mg. N. June 5-July 6, 1928*

| Date of Harvesting Seedlings | Location of Leaf on Stem, Counting from Base Upward | Total Leaf Area (sq. cm.) | Total Nitrogen (mg.) | Total Nitrogen per sq. cm. (mg.) |
|---|---|---------------------------|----------------------|----------------------------------|
| Plants grown in open in greenhouse in days of normal length | | | | |
| June 25 | 1 | 479 | 53 | 0.111 |
| | 2 | 236 | 28 | 0.121 |
| | 3 | — | 7 | — |
| | total | 715 | 88 | |
| June 29 | 1 | 479 | 38 | 0.078 |
| | 2 | 336 | 34 | 0.101 |
| | 3 | 146 | 20 | 0.137 |
| | 4 | — | 7 | — |
| | 5 | — | 1 | — |
| | total | 961 | 100 | |
| July 6 | 1 | 479 | 23 | 0.048 |
| | 2 | 349 | 28 | 0.080 |
| | 3 | 226 | 25 | 0.111 |
| | 4 | 134 | 15 | 0.111 |
| | 5 | — | 3 | — |
| | total | 1188 | 94 | |
| Seven-hour-day seedlings grown in shaded chamber | | | | |
| July 6 | 1 | 568 | 26 | 0.045 |
| | 2 | 527 | 30 | 0.057 |
| | 3 | 418 | 33 | 0.078 |
| | 4 | 323 | 25 | 0.077 |
| | 5 & 6 | 102 | 15 | 0.144 |
| | total | 1938 | 129 | |

amounts of starch, and had less development of the strengthening tissues than the stems of seedlings grown in full sunlight. The total amount of dry matter in the stems was equal to that of plants grown in the open although the percentage was less. The total amount of nitrogen found in

the stems was greater than that of seedlings grown in normal daylight, but there is some possibility that the Kjeldahl determination may not have recovered all of the nitrogen present in the stems of the latter plants.

Although an attempt was made in this experiment approximately to duplicate the light conditions in the greenhouse during December, there undoubtedly were some differences in light quality, intensity, and duration. These differences as well as those of temperature may have been responsible for such variations as were found between these shaded short-day plants grown in June and those grown during December.

Seedlings Grown in Seven-hour Day, in Shaded Chamber and Having an External Supply of Nitrogen

Figure 4, Plate XXIX, illustrates the appearance of the plants. The type of growth is remarkably like that of plants similarly grown during December and illustrated in figure 1. The dominant characteristics are the large, thin leaf blades, the long, slender, weak stems, and the very small root systems. Comparisons of the green and dry weights of the different organs with those of the December plants may be made from the data presented in table 3; similar comparisons with plants grown in normal June sunlight in days of normal length may be made from the data presented in tables 2 and 3.

The total green weight of the December plants was 400 grams and they contained 1141 mg. of nitrogen; the short day shaded plants grown in June weighed 373 grams and they contained 1091 mg. of nitrogen. The growing period in December was eleven days longer, which would more than account for the greater weight and content of nitrogen in the plants grown at that time.

DISCUSSION

The results of different investigators, some of which have been previously mentioned, have shown that the protein and chlorophyll content of leaves increases during the early phases of growth. During the adult stage fluctuations in protein content may take place. A diminution of protein and chlorophyll usually occurs in old leaves. In the studies herein described no macroquantitative determinations of the protein content of the leaves have been made but changes in the plasma content of the chloroplasts have been followed microscopically. Other investigators (3, 4, 10, 11) have shown that the chloroplasts are the parts of the leaf cell richest in protein. Since in yellowing leaves of squash seedlings the plasma content of the chloroplasts almost entirely disappears it is assumed that protein or a protein-complex is concerned to some extent if not exclusively in the changes. Although the results herein shown point to the fact that there is a close relation between the decrease of chlorophyll and the decrease of plasma from the chloroplasts, an interconnection, if it exists, cannot be shown by the methods used. It will be necessary to follow daily the changes

in chlorophyll, protein, and total nitrogen in the leaves by quantitative methods in order to obtain facts leading to a more definite answer to the question.

The results of the present investigations indicate that the changes in the yellowing leaf with respect to the destruction of protein and chlorophyll and the translocation of nitrogen from the leaf are not abrupt. There is a gradual reduction both in protein and chlorophyll content, previous to the final change. That the accumulation of carbohydrates, perhaps particularly of certain forms, may itself be a factor,³ although possibly an indirect one, in effecting the changes in the chloroplasts of squash seedlings will be shown in a following paper. The results of Smirnow's investigations suggest the possibility of a similar relationship.

SUMMARY

A. Seedlings grown without an external supply of nitrogen

1. The total green and dry weights of the seedlings increased rapidly during the first half of the developmental period when nitrogen was relatively abundant. Thereafter, the increase was less rapid. During the late phases of development there was no gain in total green weight and only a small gain in total dry weight.

2. Leaves continued to grow until after the available food reserves of the cotyledons were depleted, although at this time the leaves at the first nodes were dying and those at the second node were also showing evidences of decline. The stems elongated more during the later phases of seedling growth. The roots grew continuously until the final harvest, although the time of most rapid extension was in the early phases of seedling development.

3. Changes in type of growth and color of leaves were correlated with changes in light intensity. During the first part of the developmental period there was brilliant illumination during most of the daytime and an extensive accumulation of photosynthetic products, chiefly starch, occurred. The roots grew very rapidly during this period but the stems did not. The leaves were small and those at the first nodes were turning somewhat yellowish green. Six days of continuously cloudy weather followed. During this period features characteristic of December-grown plants were observed to develop. The leaves grew rapidly, losing all traces of their previous yellowish tint, and the stems elongated. Another period of sunshiny days followed and yellowing and decline of the older leaves again became apparent. As the older leaves were yellowing, younger

³ Another factor which influences the metabolism of the nitrogen compounds in the leaves and the withdrawal of nitrogen from the leaves is the action of some mineral available to the plants in very small amounts. There appears to be a relation between the available amounts of such a substance or substances and the accumulation of carbohydrates in the plants.

leaves were developing at higher levels on the stem. The interception of the period of cloudy weather favored the growth of the third, fourth, and fifth sets of leaves. These leaves had a considerably greater leaf area than the corresponding leaves of seedlings grown during the previous May and June when there was almost continuous sunshine during the days of the experimental period.

4. Yellowing is associated with a loss of plasma from the chloroplasts and a loss of over half of the nitrogen originally contained in the leaves. Loss of nitrogen occurs while there is some chlorophyll left in the leaf.

5. There is evidence that only a small fraction of the nitrogen released in the decomposition changes occurring in the older high-carbohydrate leaves may be reutilized by the younger growing leaves.

6. The leaves from all of the nodes exhibited a diminishing percentage content of nitrogen in their green and dry material from the early stages of development until the time of the final harvest.

7. There was a surprising constancy in the amount of nitrogen found in the roots at different stages of growth but the percentage of nitrogen in their total fresh material diminished continuously throughout their growth.

8. The absolute amount of nitrogen contained in the stems was only slightly higher at the time of the final harvest than during the intermediate phases of development. There is some possibility that the amounts found were smaller than they should have been since rather large discrepancies in the nitrogen balance were noted in the late stages of development. It seems possible that a fraction of the nitrogen may be lost as a result of respiratory processes by the leaves. It is also possible that the Kjeldahl method may fail to detect its accumulation in the stems or that a loss of nitrogen may result from the action of microorganisms in the soil.

9. There is evidence that the differences observed in growth and allocation of nitrogen in June and December plants are chiefly a result of differences in light at the two seasons. By reducing the intensity and duration of June sunlight it has been observed that:

(a) Less of the reserve nitrogen is used in the growth of roots and in the development and maintenance of leaves at the first two nodes, consequently more nitrogen is left to provide for the growth of the younger leaves.

(b) The maintenance of the chlorophyll and plasma of the chloroplasts for a longer time without degenerative effects and the smaller loss of nitrogen from the leaves cause the total nitrogen found in the leaves to be greater than that of the long-day unshaded plants. Similar light factors undoubtedly account for similar differences in the allocation of nitrogen in June and December plants.

(c) Reducing the intensity and duration of June sunlight makes possible the development of a greater leaf surface in three ways: (1) It leaves more nitrogen available for growth of leaves. (2) It causes the production of

thinner leaves with fewer cells and fewer chloroplasts per unit of surface area with the consequence that a given amount of nitrogen produces more leaf surface. (3) It appears that less nitrogen is used by adult leaves for purposes of maintenance.

(d) Reducing the intensity and duration of June sunlight results in the production of much smaller root systems but much longer weaker stems containing less reserve carbohydrates.

10. The process of carbohydrate accumulation was followed in the oldest leaves of the seedlings. Microchemical evidence indicates that when exposed to brilliant illumination there is a somewhat more rapid accumulation of starch than of reducing substances during the first half of the life of a leaf; during the latter half of its life beginning about the time at which evidences of yellowing occur, there is a large accumulation of reducing substances. As the process of yellowing advances the starch content decreases and in the later stages reducing substances also decrease. More exact quantitative methods are required to determine more precisely the relative proportions of different types of carbohydrates at different stages in the development of these leaves.

B. Seedlings grown with an external supply of nitrogen

The effect of additional nitrogen is similar to that found in previous experiments. Reducing the intensity and duration of June sunlight resulted in the production of plants which appeared to be much like those produced under normal light conditions in December.

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EXPLANATION OF PLATE XXIX

FIG. 1. Seedling grown during November-December and with additional nitrogen.

FIG. 2. Seedling grown during November-December and without additional nitrogen.

FIG. 3. Seven-hour-day seedling grown in shaded chamber in June and without additional nitrogen.

FIG. 4. Seven-hour-day seedling grown in shaded chamber in June and with additional nitrogen.

FIG. 5. Normal-length-of-day seedling grown in full daylight in June and without additional nitrogen.

FIG. 6. Normal-length-of-day seedling grown in full daylight in June and with additional nitrogen.

SOME EFFECTS OF ARTIFICIAL CLIMATES ON THE GROWTH AND CHEMICAL COMPOSITION OF PLANTS

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INTRODUCTION

Plants growing under natural conditions are affected more or less by temperature, rainfall, humidity and carbon dioxide concentration of the air, light intensity, light quality, and length of day, as well as by many soil factors. In a study of the effects of these factors on plant growth it is obviously of great advantage to have as many factors as possible under close control. One or more may then be varied in a definite direction and the effect observed on growth, flowering, dry weight increase, chemical composition, or other measurable quantity associated with the development of the plant. Unless all factors are controlled any attempt to assign measured variations in a single factor as the causative agent of a particular development of the plant would seem to be mainly speculative. Yet many of the effects on plants of variation in environmental factors are so outstanding that even with poorly controlled environmental conditions certain factors have been without doubt correctly assigned as causative agents. Such factors as temperature and light intensity, especially when decreased greatly, have such marked effects on plant development that these factors were long ago assigned as causative agents of certain growth characteristics in plants. Length of day as a causative agent in initiating flowering has more recently been separated from two closely associated factors, temperature and light intensity. By an accurate control of day length when light intensity and temperature are high, Garner and Allard (4) have shown that day length alone determines flowering in some plants. Other causative agents which produce different developmental characteristics in plants no doubt exist and will be found when natural environmental factors can be controlled with more precision.

This series of experiments includes studies made with several species of plants growing in artificial climates. The plants were grown in some experiments with artificial light only as the source of energy for photosynthesis. In other experiments they were grown with daylight supplemented by artificial light for a period of six to 12 hours each night during

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

the months of February, March, and April. An attempt was made to grow plants throughout their life history with photosynthesis at or near its maximum rate by supplying a high light intensity and long day along with increased carbon dioxid concentration and a relatively high temperature. The effect of length of day on certain species was also studied in various combinations of temperature and carbon dioxid supply. Only a few combinations of various environmental factors were tested on the various species during these experiments. Some natural climatic factors also were found to be difficult to reproduce in an artificial climate. This is especially true of sunlight. The studies therefore are mainly a preliminary survey of the effect on plant development of a number of climatic factors reproduced as accurately as possible in an artificial climate. As more efficient light sources are developed it will be possible to approximate more closely a natural environment.

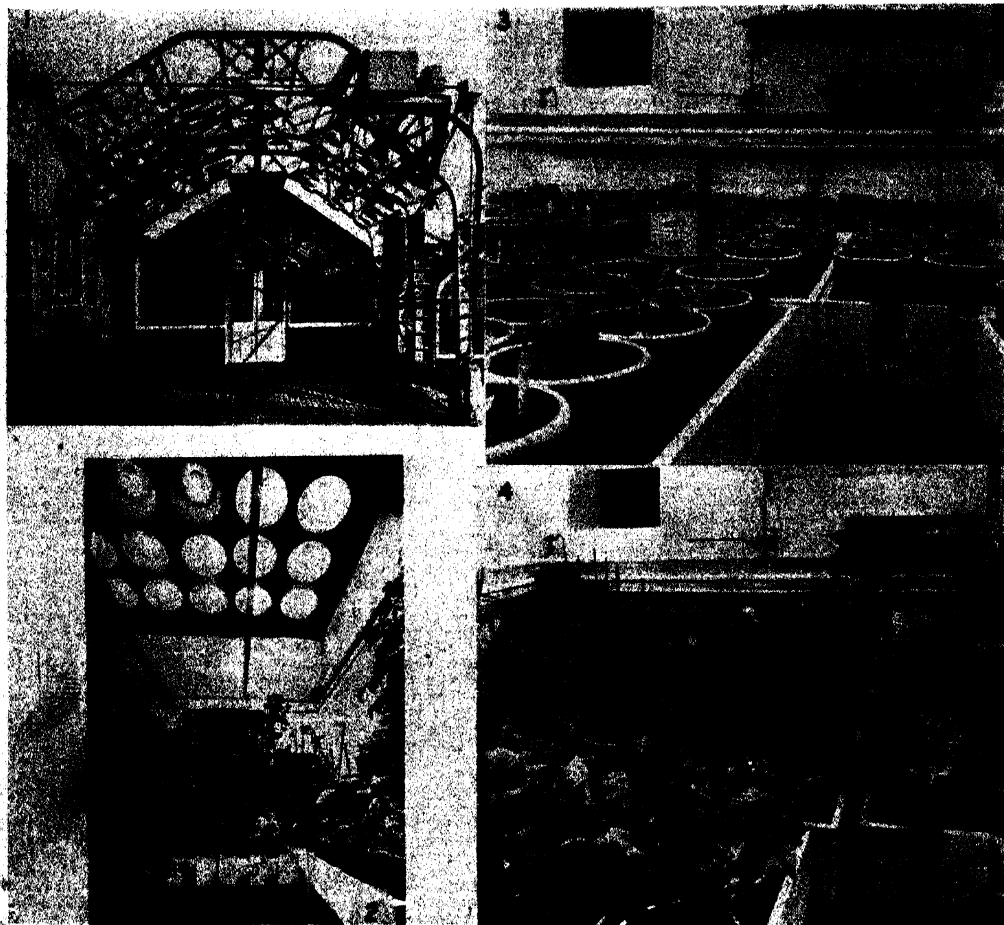
Chemical analyses of many plants grown under the different conditions are given together with a discussion of the effect of various factors on percentage carbohydrate and nitrogen in various tissues.

APPARATUS AND EQUIPMENT

Two kinds of equipment were used in these experiments. The first was the gantry crane greenhouses where sunlight was used during the day and a battery of 48 1000-watt lamps carried on the crane supplemented daylight for six to 12 hours each night. A photograph of the crane is reproduced in text figure 1. The second type of equipment was the constant condition rooms where plants were grown entirely with artificial light furnished mainly by a battery of 25 1000-watt lamps. The constant-light room is illustrated in text figure 2. A more detailed description of each of the two types of equipment follows.

Gantry Crane Greenhouses

The gantry crane greenhouses consisted of a set of two houses each 26×20 feet placed end to end with a four-foot vestibule between. The vestibule was ventilated independently and served to check the diffusion of carbon dioxid into the house having only the normal concentration of this gas. Each house contained two benches running parallel to the ridge and arranged to support jars of growing plants at a height of 56 inches above the floor level. Each bench was approximately $6\frac{1}{2} \times 20$ feet. They were spaced 30 inches apart so as to leave an aisle of this width running through the center of each house parallel to the ridge. The crane was built to travel upon iron rails placed on either side of the two greenhouses. It was driven into place over one of the greenhouses by means of an electric motor, where it remained for a period of six to 12 hours each night. It was removed each morning so as to avoid shading. The crane carried 48 1000-watt incandescent filament lamps arranged in rows so that



TEXT FIG. 1. Gantry crane carrying 48 1000-watt lamps used as a light source in the greenhouse to supplement daylight for 6 to 12 hours each night. TEXT FIG. 2. Plants growing with artificial light only in the constant-light room. This picture shows the position of the glass-water filter between the lamps and the plants. 25 1500-watt lamps were used as a light source. TEXT FIG. 3. A photograph of plants growing in the constant-light room four days after the start of the 1925 experiment. TEXT FIG. 4. Same as FIG. 3 except taken 17 days later. The lettuce plant on Chart 6 B (19 hour day) was grown in 17 days from the small plant similarly located in FIG. 3. The buckwheat plants on a 24 hour day in the rear of the date card grew to a height of 24 inches in 17 days. These plants were about 2 inches high in FIG. 3.

each half of the greenhouse roof was illuminated by 24 1000-watt lamps. The lamps were fitted with mirror reflectors and were arranged to distribute the light uniformly over each of the two benches with a minimum intensity over the walks in the center of the house. In the first experiments, 1924 and 1925, lamps were used with a rated voltage of 115 to 120. The current supplied was 108 volts at the main switchboard but there was a voltage drop toward the socket so that the measured voltage at the socket was only 105. In later experiments the rated voltage of the lamps was 105. This results in a greatly increased intensity and efficiency of the lamps. All lamps were replaced at the end of each experiment and it was found that a lamp with a rated voltage approximately identical with the socket voltage would maintain well the initial light output during the time of the experiment. Experiments in general lasted 8 to 10 weeks, during which time the lamps burned a total of 700 to 850 hours. It should be pointed out that these lamps are designed to be burned for 1000 hours and that unless current is very cheap it does not pay to burn them longer since the light output falls rapidly after this life span has been reached.

In the first experiments air temperature in the gantry crane houses was controlled by thermostats operating steam control valves by means of compressed air. These were not dependable unless supplemented by hourly inspection, and they were later replaced by electrically operated solenoid valves. This gave a very positive control of the steam supply for heating and by hourly inspection during warm weather the greenhouse vents could be kept open far enough to keep the temperature down. Temperatures were recorded continuously during all experiments by means of recording galvanometers connected with electrical resistance thermometers placed in each house. In general, temperatures were held within a plus or minus variation of approximately three degrees.

The control plants were kept in a greenhouse similar in dimensions to the gantry crane houses. Here, however, a slightly better control of air temperature and humidity was obtained by recirculating the air by means of a standard air-conditioning system. The operation of this system depends upon first, the saturation of the air coming from the greenhouse with a thermostatically controlled mixture of cold water and steam; and second, the re-heating of the air in the ducts as it rises toward the greenhouse to bring it to the desired temperature. Depending upon the temperature of saturation and the temperature of re-heating a definite degree of relative humidity can be maintained within certain limits. In most of the experiments reported herewith the thermostats were set to maintain a relative humidity of 80 percent.

Constant Condition Rooms

These rooms are equipped for growing plants entirely with artificial light. They are two in number, the constant-light room and the adjoining dark room. Each room is approximately 11 feet square and is located in

the basement under the greenhouses. The rooms are connected by a short closed corridor, and in order to obtain different lengths of day and night plants may be moved from the light to the dark and back again with no temperature change and the minimum of shock. The same air was recirculated through both rooms by means of standard air-conditioning machinery similar to that already described in connection with the control greenhouses. The only difference was that ice water supplied by a 15-ton ice machine was used, while the amount of cooling in case of the control greenhouse was limited to the temperature of tap water. Air temperature in the light and dark room was maintained accurately within a plus or minus range of one degree centigrade, and humidity controlled to a plus or minus three percent relative during the whole growth period of the plants. Wet and dry bulb temperatures were recorded by means of recording galvanometers in connection with resistance thermometers.

The main light source in the constant-light room was from 25 1500-watt gas-filled tungsten filament lamps suspended from a metal frame in the ceiling of the room. The lamps were fitted with Reflectors and Light Manufacturers Standard Reflectors and were arranged to give uniform illumination on the benches where the plants were grown. A false ceiling of clear plate glass was built between the lamps and the growing plants. This was fitted with a weir at one end which was adjustable in height. Water was fed in at the opposite end and the weir was set to maintain a layer of water $\frac{1}{4}$ to $\frac{1}{2}$ inch in thickness over the entire surface of the glass plate. This glass-water filter served to absorb some of the infra-red output of the lamps. It also resulted in a considerable loss of energy in the visible region. This was especially true when dust and green algae were allowed to accumulate. Algae gave a great amount of trouble since such conditions of high temperature and high light intensity are ideal for their growth. It was found that a cheesecloth bag filled with zinc oxid and suspended in the water supply served to check the growth of algae. This treatment supplemented by a thorough scrubbing of the glass surface every other day maintained fairly well the initial transmission of the glass-water filter.

A motor driven fan was used to supply forced ventilation to the space around the lamps and above the glass-water filter. This also served to get rid of the large amount of excess heat from the lamps. This fan motor switch was interlocked with the switches supplying power to the lamps so that the lamps could not be burned until the fan motor was running.

In the first experiment in 1924 the glass-water filter was 40 inches from the tips of the lamps. The distance from the filter to the soil in which the plants grew was approximately 65 inches, making a total distance from the lamp to the soil of about 105 inches. In later experiments the lamps were moved down toward the filter so that in the last experiments (1926 and 1927) the lamps were only 19 inches above the surface of the water, or 84 inches above the soil. Since plant growth was very rapid the distance between

the tip of the plant and the filter rapidly decreased with a resulting very slight increase in light intensity. Some of the taller plants such as sunflower, corn, and buckwheat grew sufficiently to touch the filter before the experiment was closed.

The resulting light on the soil in which the plants were grown was measured by both the Macbeth Illuminometer and a pyrheliometer described by Kimball and Hobbs (10) and in use at various Weather Bureau stations for recording solar intensity. These instruments will be discussed later. The pyrheliometer was connected with a recording millivoltmeter and calibrated in energy units. These data are also tabulated later. In the first experiment of 1924 light intensity decreased until at the end of the experiment it had fallen to about 50 percent of the original value. This was found to be due to the aging of the lamps which usually occurs after about 40 days of continuous burning. In all later experiments lamps were replaced or the experiment discontinued after 45 days.

Carbon Dioxid Supply

In one of the gantry crane houses and in the constant-light room the carbon dioxid concentration was maintained at ten times the normal, or about 0.3 percent. The carbon dioxid was supplied from steel cylinders holding 50 pounds of this gas. Three cylinders were connected to a manifold at the same time. The gas was first heated as it left the tanks by means of a coiled tube immersed in hot water. This treatment prevented freezing which would otherwise occur due to the rapid expansion of the gas. It was then expanded through a reducing valve into a 30-gallon cushion tank which insured an even flow through the gas meters located in the lines which delivered the gas to the growing houses. These meters were identical with the household type which public service companies use to meter illuminating gas except that they were fitted with a larger dial for more accurate reading. After a few determinations by gas analysis of the number of revolutions per minute necessary to maintain the concentration desired the valves were opened so as just to maintain this rate on the meters. In the 1926 and 1927 experiments a low range carbon dioxid recorder made by the Leeds and Northrup Company was used. This recorder had a range of 0 to 3.5 percent. It has been described by Rosecrans (17).

Approximately six cylinders of carbon dioxid or 300 pounds of the gas were required in each 24-hour period to maintain a concentration of about 0.3 percent in one gantry crane house and in the constant-light room. When the greenhouse vents at both the ridge and eaves were opened to keep temperature down even this rapid flow of gas failed to maintain the desired concentration. It should be stated here that it is almost impossible to maintain any appreciable concentration of carbon dioxid in a greenhouse in this way on account of very rapid convection currents which sweep the gas out as fast as it is delivered.

A separate experiment was made in 1926 to determine whether one percent carbon dioxide would produce more growth than 0.3 percent during the months of October, November, and December without additional light. It was found that a great number of plants produced more weight when grown in 0.3 percent concentration of this gas but that one percent gave no further increase. The higher concentration produced no injury, but it was much more expensive and difficult to maintain so that the lower concentration of 0.3 percent was used in all other experiments.

In 1926 and 1927 a method for producing carbon dioxide from flue gases was studied. In the results of experiments presented here data concerning plants grown with carbon dioxide from this source are listed under the caption "Flue gas" or sometimes in the case of photographs, "F.G." Gases arising from the burning of anthracite coal were treated in the following way. Hot gases were pumped by means of a negative pressure fan from the stack immediately above the boiler, first through a steel scrubbing tower where they were atomized with water. The gases were cooled and partially washed in this tower. They passed on through a similar tower where they were atomized with a one percent potassium permanganate solution. The gases then passed into a third tower where they were washed again with water and thoroughly saturated before moving through four filter cabinets. The first three filter cabinets were filled with trays of sawdust and sphagnum moss while the last one contained trays of glass wool which removed the last traces of finely divided carbon. The function of the permanganate solution is not definitely known. It has been found by trial, however, that it oxidizes certain compounds in the gases which are otherwise harmful to plants. It is slowly oxidized so that the solution becomes completely decolorized in about three to four weeks when the apparatus is running continuously for nine hours each day. The permanganate solution returning from the tower is collected in a 300-gallon earthenware jar and re-circulated by means of a small pump. The third tower serves to wash out any permanganate carried over and effectively increases the size of the finely divided soot particles so that all traces of soot are removed by the filters. This is especially important when the gases are used in greenhouses since any finely divided carbon brought in by the gases is precipitated on the cold surface of the glass as well as on plants and in time builds up a sooty, black deposit. The present filtering arrangement has been used to produce gas for a single greenhouse during two seasons of growth in the winter months and has caused no appreciable blackening of the glass or white framework during this time.

Light Intensity Measurements

During the 1924 experiments light intensity was measured only by a Macbeth illuminometer. In all later series both the illuminometer and a recording thermo-electric pyrheliometer were used. The former instrument

is a portable photometer carrying a lamp which is calibrated so as to give a known illumination value with a definite current. It is especially useful in measuring the illumination of artificial light sources indoors. On account of the color difference between the artificial light source in the instrument and sunlight it is more difficult to measure solar intensities. There is also no relation between the reading of an illuminometer in foot candles and the radiant energy received in gram calory units which is applicable to all light sources since the illuminometer is concerned only with visible radiation. A factor can be obtained, however, for a given artificial light source operating under known conditions which will allow the conversion of the reading of an illuminometer in foot candles to energy units as determined by a pyrheliometer or other apparatus for measuring radiant energy. A 1000-watt lamp fitted with a standard reflector at 37.5 inches gave an illumination value of 620 foot candles as measured by an illuminometer or 0.3 of a gram calory per square centimeter per minute as measured by the pyrheliometer. The factor in this case is approximately 2100. This factor is also approximately the same as that obtained in the gantry crane houses. Ageing of the lamps decreases the value while the effect of a glass water screen as used in the constant-light room is to increase it greatly due to absorption of the infra-red region with very little loss in illumination value. In the case of the constant-light room the factor is approximately 5000, except in 1925 when the line voltage was too low to operate the lamps efficiently. Kimball (9) has found a similar illumination equivalent for sunlight which gives the illumination value in foot candles from the energy value as determined by the pyrheliometer, this value being 6700 for cloudless skies and 7000 for a sky covered with clouds. The value as determined in the constant-light room approaches that for sunlight. While this factor approaches the value 6700 found by Kimball for solar radiation it does not indicate that the light in the case of the constant-light room was equal to solar radiation in spectral energy distribution. The approximate spectral radiation components of four different light sources similar to those used in this work as published by Coblentz, Dorcas, and Hughes (3) are as follows:

| | In percentage of the total radiation to 12,000 ft. | | |
|---|---|-----------------|------------------|
| | 450-600 $m\mu$ | 600-1400 $m\mu$ | 1400-4200 $m\mu$ |
| Sun, Washington, D. C. | 22.0 $m\mu$ | 39.5 $m\mu$ | 19.7 $m\mu$ |
| Air mass 1.3, July 28, 1926, 11 A.M. | 6.7 " | 3.2 " | 20.5 " |
| Quartz mercury arc. | 3.8 " | 29.8 " | 54.2 " |
| Gas filled tungsten lamp, 12.7 amps. | 9.8 " | 19.5 " | 39.2 " |
| White flame carbon arc 30 amps. | | | |

COLLECTION AND TABULATION OF DATA

Weekly measurements of growth in height were made and any development of buds, flowers, and fruit or changes in foliage color were noted.

These measurements were supplemented by frequent photographs, several of which are reproduced along with the chemical data.

Chemical Analysis ²

Many plants grown under the various conditions were sampled and analyzed for various carbohydrate and nitrogen constituents. The sampling was usually done toward the end of an experiment when the plants were nearing maturity except in certain cases where an attempt was being made to determine the effect of age on these constituents. Sampling was done at the end of the period of exposure to light in every case except where otherwise noted. In the case of those plants growing in the gantry crane greenhouses which were exposed to daylight plus artificial light from 6 P.M. until midnight, the plants were harvested at midnight and placed in the cold room at a temperature near zero degrees Fahrenheit where they remained until they were ground and preserved in alcohol on the following day. Plants could be kept at this temperature for 24 to 48 hours with very little change in carbohydrate fractions. Because of the difficulty in getting plant roots free from soil only the aerial portion in general was sampled. There were a few exceptions to this procedure in the case of those plants having large fleshy roots. The tissue was ground with a Russwin steel knife mill and divided into duplicate or triplicate samples for analysis. Separate samples were also taken for moisture determinations. The samples for analysis were placed in 300-cc. Erlenmeyer flasks and weighed. One-tenth gram of calcium carbonate was added and the flasks were filled two-thirds full of boiling 95-percent alcohol and boiled for ten minutes. They were then filled to the neck with alcohol and stoppered and placed in storage until the tissue could be conveniently analyzed.

In general the official methods for feeding stuffs published by the American Association of Official Agricultural Chemists was followed. The tissue was extracted with 50-percent alcohol. Reducing sugars were determined on the cleared extract and the fraction listed as sucrose determined by reduction, after hydrolysis of this extract, by subtracting the value for reducing sugars first obtained. The acid hydrolyzable fraction was determined as reducing substances in the hydrolyzed and cleared residue. Hydrolysis was accomplished by heating for two and one-half hours with 20 cc. of hydrochloric acid, specific gravity 1.125, and 200 cc. of water in a flask fitted with a reflux condenser. The results are calculated as dextrose and multiplied by the factor .9.

Soluble nitrogen was determined from an aliquot of the alcoholic extract and insoluble nitrogen from an aliquot of the dried residue by the Kjeldahl method modified to include nitrates.

The amount of moisture was determined by drying duplicate samples of the ground tissue in a vacuum oven at 70° C.

² The writers are indebted to Dr. J. E. Webster for analyses of plants grown in the 1924 series.

TABLE I. *Effect on Chemical Constitution of Keeping Tomato Plants in Darkness for Various Periods Before Sampling. Whole Aerial Portion*

| Growth Conditions | Weight per Plant (Grams) | Moisture % | Nitrogen, % Dry Weight | | Carbohydrate, % Dry Weight | | | | Carbohydrate Nitrogen |
|---|--------------------------------|---------------|---------------------------|-------|----------------------------|---------|----------|-------|--------------------------|
| | | | Soluble | Total | Acid Hydro- lyzable | Sucrose | Dextrose | Total | |
| 1. Exposed July 6 to 30, 1928, to sunlight. Leaves only | | | | | | | | | |
| 1. Greenhouse, sampled at 3 P.M..... | 15 | 83.0 | .24 | 2.42 | 33.7 | 1.41 | 2.94 | 38.1 | 15.8 |
| 2. Same except after 17 hours in darkness.. | 16 | 84.2 | .25 | 2.53 | 29.9 | .57 | 1.71 | 32.2 | 12.8 |
| 3. Grown outdoors, sampled at 3 P.M..... | 10 | 82.7 | .29 | 2.83 | 26.8 | 1.45 | 3.35 | 31.6 | 11.2 |
| 4. Same as 3 except after 17 hours in dark- ness..... | 10 | 84.3 | .19 | 2.87 | 26.4 | .57 | 2.04 | 29.0 | 10.5 |
| Stems only | | | | | | | | | |
| 5. Same as 1..... | 19 | 88.5 | .26 | 1.13 | 15.0 | 4.52 | 5.83 | 25.4 | 22.4 |
| 6. Same as 2..... | 24 | 89.1 | .18 | 1.01 | 14.3 | 2.84 | 6.06 | 23.2 | 23.0 |
| 7. Same as 3..... | 12 | 87.0 | .23 | 1.08 | 13.3 | 4.38 | 6.62 | 24.3 | 22.5 |
| 8. Same as 4..... | 14 | 88.9 | .27 | 1.17 | 14.5 | 2.61 | 6.22 | 23.3 | 19.5 |
| 2. Exposed to artificial light, 700 f.c. without filter April 30 to May 4 as compared to greenhouse plants. Leaves only | | | | | | | | | |
| 9. Greenhouse, sampled at 3 P.M..... | 15 | 86.3 | .29 | 3.19 | 23.9 | 1.49 | 2.00 | 27.4 | 8.6 |
| 10. Same except sampled in early A.M..... | 16 | 86.7 | .30 | 3.58 | 17.5 | .26 | 1.48 | 19.2 | 5.4 |
| 11. Artificial light, sampled at once..... | 15 | 85.7 | .29 | 2.78 | 28.0 | 1.11 | 2.25 | 31.3 | 11.2 |
| 12. Same except sampled after 40 hours darkness..... | 16 | 88.9 | .58 | 3.85 | 9.5 | 0.0 | 1.20 | 10.7 | 2.78 |
| Stems only | | | | | | | | | |
| 13. Same as 9..... | 26 | 91.7 | .30 | 1.14 | 13.9 | 2.74 | 5.63 | 22.3 | 19.6 |
| 14. Same as 10..... | 22 | 91.3 | .28 | 1.09 | 13.0 | 2.22 | 6.14 | 21.3 | 19.5 |
| 15. Same as 11..... | 30 | 91.4 | .46 | 1.37 | 14.0 | 2.17 | 7.14 | 23.3 | 17.0 |
| 16. Same as 12..... | 34 | 92.4 | .71 | 1.55 | 12.3 | 1.01 | 5.03 | 18.3 | 11.8 |

Effect of Darkness on Carbohydrate Fractions

A series of analyses was made on tomato plants exposed in one case to daylight and in another to artificial light and then kept in darkness at room temperature for various periods before sampling. This study was made primarily to determine the magnitude of the decrease in carbohydrate fractions during short periods of darkness. These periods are comparable to the effect of night and periods of very low light intensity which plants often receive when growing under natural conditions. The data are given in table 1. It will be observed from the data given that plants gain slightly in percentage moisture during such periods of rest. Nitrogen fractions are not changed appreciably. Total carbohydrates in general decrease slightly after 17 hours of darkness while after 40 hours this value has decreased to about one-third of the original figure. The greatest decrease is in the leaves in the shorter periods of darkness, while in prolonged darkness even the stems are greatly depleted in easily available carbohydrates. There is a difference in the percentage carbohydrate in plants sampled in the evening after a day of rapid photosynthesis as compared with plants sampled in the morning before any photosynthesis takes place. The effects of these rapid changes in carbohydrates are reflected in the carbohydrate-nitrogen ratio as indicated in the last column of table 1. It is seen that darkness causes wide variations in this relation so that a combination of light and darkness can be chosen to produce in a few hours either a high or low carbohydrate plant depending upon the time the plant is sampled relative to periods of light and darkness. These changes are accomplished through an alteration of the percentage of carbohydrate in the whole plant with very little change in the percentage of nitrogen. The carbohydrate-nitrogen ratio will be discussed later in greater detail.

Errors of Sampling

In order to gain some idea of the individual errors of sampling, two species used extensively in these studies were selected for a detailed study of sampling errors. One was the aerial portion of radish and the other the tomato plant. A large number of plants of each species were grown under similar conditions. They were then sampled in smaller separate groups. These data are included in tables 2 and 3. Table 3 shows both the variation in duplicate samples and that of individual plants. By comparing the figures in each column with the average at the foot of the respective columns it will be observed that the sampling errors are relatively small. Groups of individuals from both species of plants when grown under the same conditions vary but little in chemical constituents.

CONDITIONS OF EXPERIMENTS

Experiment 1. June 3 to August 15, 1924

Constant-light room, artificial light only.

Temperature 78° F.

TABLE 2. Analyses of Special Radish Tops Grown in Control. Seed Planted Feb. 12 and Plants Sampled March 8, 1927

| Sample | Total Wt. of Tops | Wt. of Tops per Plant | Moisture | Nitrogen | | | | | | | | Acid Hy- drolyzable Material | | Sucrose | | Dextrose | | Total Carbohy- drates | |
|---------|-------------------------|-----------------------------|----------|-----------|------|---------|------|-------|------|-------|-------|------------------------------------|-------|---------|-------|----------|-----|-----------------------------|-----|
| | | | | Insoluble | | Soluble | | Total | | | | | | | | | | | |
| | | | | Green | Dry | Green | Dry | Green | Dry | Green | Dry | Green | Dry | Green | Dry | Green | Dry | Green | Dry |
| R 1 | 82 | 3.0 | 92.38 | 0.30 | 3.95 | 0.15 | 1.97 | 0.45 | 5.92 | 0 | 0 | 0.14 | 1.82 | 0.67 | 8.81 | | | | |
| R 2 | 89 | 3.1 | 92.48 | 0.29 | 3.81 | 0.16 | 2.19 | 0.45 | 6.00 | 0 | 0 | 0.15 | 2.04 | 0.72 | 9.58 | | | | |
| R 3 | 77 | 2.8 | 91.47 | 0.30 | 3.49 | 0.17 | 2.03 | 0.47 | 5.52 | trace | trace | 0.16 | 1.83 | 0.68 | 7.98 | | | | |
| R 4 | 63 | 2.1 | 92.23 | 0.28 | 3.60 | 0.17 | 2.27 | 0.45 | 5.87 | 0.10 | 1.33 | trace | trace | 0.64 | 8.28 | | | | |
| R 5 | 59 | 2.2 | 92.38 | 0.28 | 3.68 | 0.15 | 1.92 | 0.43 | 5.60 | 0.23 | 2.95 | trace | trace | 0.81 | 10.55 | | | | |
| R 6 | 50 | 1.6 | 90.63 | 0.38 | 4.07 | 0.23 | 2.43 | 0.61 | 6.50 | 0.16 | 1.68 | 0 | 0 | 0.80 | 8.54 | | | | |
| R 7 | 69 | 1.9 | 91.85 | 0.29 | 3.55 | 0.14 | 1.75 | 0.43 | 5.30 | 0.24 | 2.99 | trace | trace | 0.81 | 10.00 | | | | |
| R 8 | 64 | 2.0 | 91.25 | 0.34 | 3.84 | 0.21 | 1.23 | 0.55 | 5.07 | 0.07 | 0.81 | 0.11 | 1.23 | 0.79 | 9.00 | | | | |
| R 9 | 64 | 2.2 | 91.13 | 0.34 | 3.85 | 0.22 | 2.48 | 0.56 | 6.33 | 0.17 | 1.93 | trace | trace | 0.77 | 8.67 | | | | |
| R 10 | 80 | 2.8 | 91.70 | 0.31 | 3.75 | 0.15 | 1.84 | 0.46 | 5.59 | 0.15 | 1.82 | 0.12 | 1.40 | 0.87 | 10.43 | | | | |
| R 11 | 71 | 2.4 | 92.24 | 0.24 | 3.08 | 0.16 | 2.03 | 0.40 | 5.11 | 0.12 | 1.52 | 0.08 | 1.09 | 0.77 | 9.99 | | | | |
| Average | | | 91.79 | 0.34 | 3.69 | 0.17 | 2.01 | 0.48 | 5.71 | — | — | — | — | 0.76 | 9.25 | | | | |

TABLE 3. Duplicate Analyses of Tomato Plants to Show Individual Variation. Grown Under a Glass which Transmits Only 40 Percent of Sunlight. 1927. Whole Aerial Portion

| Sample | Total Wt. of Plant | Moisture | Nitrogen | | | | | | Acid Hy- drolyzable Material | | Sucrose | | Dextrose | | Total Carbohy- drates | |
|---------|--------------------------|----------------|-----------|------|---------|------|-------|------|------------------------------------|-------|---------|------|----------|------|-----------------------------|-------|
| | | | Insoluble | | Soluble | | Total | | | | | | | | | |
| | | | Green | Dry | Green | Dry | Green | Dry | Green | Dry | Green | Dry | Green | Dry | Green | Dry |
| 0224 | 472 | 91.80 | 0.180 | 2.21 | 0.148 | 1.82 | 0.328 | 4.03 | 0.872 | 10.70 | 0.146 | 1.79 | 0.288 | 3.53 | 1.346 | 16.02 |
| | | 91.89 | 0.182 | 2.23 | 0.142 | 1.74 | 0.324 | 3.97 | 0.862 | 10.58 | 0.145 | 1.78 | 0.284 | 3.48 | 1.291 | 15.84 |
| 096 | 414 | 90.77 | 0.178 | 1.93 | 0.185 | 2.01 | 0.363 | 3.94 | 0.991 | 10.76 | 0.238 | 2.58 | 0.398 | 4.32 | 1.627 | 17.66 |
| | | 90.80 | 0.178 | 1.93 | 0.193 | 2.10 | 0.371 | 4.03 | 0.966 | 10.48 | 0.256 | 2.78 | 0.382 | 4.15 | 1.604 | 17.41 |
| 0215 | 690 | 91.55 | 0.165 | 1.95 | 0.157 | 1.86 | 0.322 | 3.81 | 0.856 | 10.13 | 0.133 | 1.57 | 0.334 | 3.95 | 1.323 | 15.65 |
| | | 91.53 | 0.169 | 2.00 | 0.161 | 1.90 | 0.330 | 3.90 | 0.881 | 10.40 | 0.149 | 1.76 | 0.319 | 3.77 | 1.349 | 15.93 |
| 093 | 958 | 92.53 | 0.165 | 2.21 | 0.127 | 1.70 | 0.292 | 3.91 | 0.805 | 10.81 | 0.120 | 1.66 | 0.165 | 2.21 | 1.090 | 14.63 |
| | | 92.57 | 0.159 | 2.13 | 0.130 | 1.74 | 0.289 | 3.87 | 0.829 | 11.13 | 0.112 | 1.50 | 0.147 | 1.97 | 1.088 | 14.60 |
| 500 | 510 | 92.17 | 0.169 | 2.16 | 0.143 | 1.82 | 0.312 | 3.98 | 0.756 | 9.64 | 0.145 | 1.85 | 0.265 | 3.38 | 1.166 | 14.87 |
| | | 92.15 | 0.163 | 2.08 | 0.147 | 1.88 | 0.310 | 3.96 | 0.798 | 10.18 | 0.137 | 1.75 | 0.239 | 3.05 | 1.174 | 14.98 |
| 0191 | 398 | 90.79 | 0.194 | 2.12 | 0.158 | 1.73 | 0.352 | 3.85 | 1.081 | 11.84 | 0.124 | 1.36 | 0.379 | 4.15 | 1.584 | 17.35 |
| | | 90.95 | 0.198 | 2.17 | 0.156 | 1.71 | 0.354 | 3.88 | 0.957 | 10.48 | 0.170 | 1.86 | 0.340 | 3.72 | 1.467 | 16.06 |
| 1072 | 684 | 92.22 92.17 | 0.171 | 2.19 | 0.138 | 1.77 | 0.309 | 3.96 | 0.731 | 9.37 | 0.154 | 1.97 | 0.261 | 3.35 | 1.146 | 14.69 |
| 403 | 457 | 91.50 | 0.198 | 2.35 | 0.156 | 1.85 | 0.354 | 4.20 | 0.826 | 9.80 | 0.109 | 1.29 | 0.317 | 3.64 | 1.252 | 14.73 |
| | | 91.63 | 0.192 | 2.28 | 0.135 | 1.60 | 0.327 | 3.88 | 0.808 | 9.58 | 0.133 | 1.58 | 0.293 | 3.48 | 1.234 | 14.64 |
| Average | | 91.5 | 0.184 | 2.11 | 0.151 | 1.82 | 0.328 | 3.95 | 0.871 | 10.00 | 0.151 | 1.81 | 0.294 | 3.50 | 1.310 | 15.70 |

Humidity 80 percent.

Carbon dioxid concentration 0.1 to 0.8 percent; 4 50-pound tanks used in each 24 hours.

Light exposures of 5, 7, 12, 17, 19, and 24 hours in each 24 hour period.

Light source 25 1000-watt 120-volt lamps operating on 105-volt supply.

Light filter one-half inch of plate glass plus one inch of water.

Average Macbeth readings at soil level: 450 foot candles.

Experiment 2. August 28 to October 15, 1924

Same as Experiment 1 except no additional carbon dioxid.

Average Macbeth reading 350 foot candles.

Experiment 3. February 28, to May 14, 1925

1. Gantry crane House 1, daylight supplemented with artificial light from midnight until 6 A.M. each night, normal concentration of carbon dioxid. House 2, daylight supplemented with artificial light from 6 P.M. until midnight and with carbon dioxid concentration at about 0.3 percent or ten times the normal.

Light source 48 1500-watt 120-volt lamps operating on 105-volt current.

Average pyrrehliometer reading: 0.45 gram calory per square centimeter per minute.

Equivalent in foot candles: 819.

Temperature in all houses 78° F.

2. Constant-light room, artificial light only.

Carbon dioxid concentration about 0.3 percent.

Light source 25 1500-watt 120-volt lamps operating on 105-volt current.

Light filter of plate glass with water, average depth three-eighths inch.

Average pyrrehliometer reading: 0.3 gram calory per square centimeter per minute.

Macbeth reading: 800 foot candles.

Temperature 78° F.

Humidity 80 percent.

Light exposures of 5, 7, 12, 17, 19, and 24 hours in each 24 hour period.

Experiment 4. February 28 to May 8, 1926

1. Gantry crane houses same illumination as 1925 except 48 1000-watt 105-volt lamps operating on 105-volt current supply.

Average pyrrehliometer reading: 0.36 gram calory per square centimeter per minute.

Macbeth reading: 760 foot candles.

House 1, daylight plus gantry crane illumination from midnight until morning.

House 2, daylight plus gantry crane illumination from 6 P.M. until midnight and with ten times the normal carbon dioxid concentration.

Temperature 68° F. in all houses.

Humidity 80 percent in control house.

2. Constant-light room; artificial light only.

Carbon dioxid concentration ten times normal.

Light source 25 1500-watt 105-volt lamps operated on 104-volt current.

Light filter same as Experiment 3.

Four mercury vapor arcs in glass tubes.

Average pyrhelimeter reading: 0.25 gram calory per square centimeter per minute.

Macbeth reading: 1200 foot candles.

Temperature 68° F.

Relative humidity 80 percent.

Light exposures of 5, 7, 12, 17, 19, and 24 hours in each 24 hour period.

Experiment 5. January 28 to April 8, 1927

1. Gantry crane houses same illumination as in 1926 except only one house, number 2, illuminated.

House 2, daylight 12 hours plus gantry crane 12 hours from 6 P.M. until 6 A.M. making a 24 hour day.

Carbon dioxid ten times normal.

Temperature 78° F. except in a small vestibule held at 68° F.

Relative humidity in control house 90 percent.

2. Constant-light room, artificial light only.

Carbon dioxid concentration ten times normal.

Light source 22 1500-watt 105-volt lamps operated on 105-volt current.

Three 25 amperes white flame carbon arcs. Two mercury vapor arcs in glass tubes.

Light filter same as experiment 3.

Average pyrhelimeter reading: 0.24.

Macbeth reading: 1400 foot candles.

Temperature 68° F.

Relative humidity 90 percent.

RESULTS OF EXPERIMENTS

More than thirty different species of plants were grown in these experiments. It was found impossible to get a complete set of data on the whole 30 species as regards chemical analysis and day-length effects. A few representative species of special interest were therefore selected for a more detailed study. These have been grown with different day-lengths from five hours to 24 in the constant-light room and also with daylight supplemented by six to 12 hours each night with artificial light from the gantry crane.

Plants in general were found to increase greatly the weight of tissue produced when given daylight plus six hours additional light each night. A still further increase was produced by increasing carbon dioxid concen-

tration along with supplementary lighting. Many plants grew very rapidly in the constant-light room with artificial light only. Text figures 3 and 4 show 17 days' growth under these conditions. The large lettuce plant shown in the foreground of figure 4 was produced in this time period from the small plant similarly located in figure 3 and illustrates the rapid growth under these conditions. When grown with artificial light the weight of tissue produced increases with day length up to approximately an 18 hour day while there is no corresponding increase when given a 24 hour day. Some species were found to grow as well with continuous 24 hour illumination as on an 18 hour day, while others were found to be greatly injured by a 24 hour day. The results of the chemical analyses of various species as related to carbohydrate-nitrogen ratio, injury of long day, and specific effects of the various conditions on a number of plants will be discussed in the following pages.

Carbohydrate-nitrogen Content as Related to Flowering and Length of Day

Considerable study has been made of the effect of various day lengths on the carbohydrate-nitrogen relations of various plants grown in this series of experiments. The study has been especially directed toward representative varieties of each type of plant as originally worked out by Garner and Allard (4), the short and long day and the everblooming types. Both radishes and lettuce are of the long day type and both grow well up to a 24 hour day or continuous illumination. They were therefore selected as suitable plants for growing on a number of day lengths from five to 24 hours. Similarly, salvia was selected as a typical short day plant and buckwheat as an everblooming type. Salvia flowers well on day lengths of 15 hours or shorter while buckwheat flowers equally well on all day lengths from five hours to 24. In this series of experiments it was hoped to establish a possible relation between the percentage composition of carbohydrate and nitrogen constituents and the day length at which the plants would flower. Assuming that day length determines flowering in these species through a building up of a certain amount of carbohydrate with relation to the total amount of nitrogen, one should be able to determine how much of each fraction is necessary before flowering is initiated in the long day plant and what carbohydrate maximum just prevents flowering in the short day plant. This premise also assumes that the amount of photosynthetic material manufactured is in some simple proportion to the dosage of light, that is an intensity \times the time of exposure, within certain limits. Where more carbohydrates are built up with greater dosage of light it is reasonable to expect that translocation and further synthesis will not be increased at the same rate, consequently carbohydrates will increase to a greater or lesser degree, with increasing day length, if intensity remains the same. If the ratio of total carbohydrate to total nitrogen is effective in determining flowering it should be possible to regulate this process



TEXT FIG. 5. Lettuce plants grown on 5, 7, 12, 17, 19, and 24 hour days in the constant-light room, showing the flowering on day lengths of 17, 19, and 24 hours. TEXT FIG. 6. *Salvia* plants flowering on short day lengths of 5, 7, and 12 hours. Only an occasional terminal flower is produced on a 17 hour day. This plant flowers well on all day lengths from 5 to 15 hours. TEXT FIG. 7. Buckwheat plants grown in constant-light room. This plant flowers on all day lengths from 5 to 24 hours. The height growth increases with day length up to 19 or 24 hours. The control plants were grown in the greenhouse. The plants are all 32 days old from seed. TEXT FIG. 8. Tomato plants from constant-light room grown on 5, 7, 12, 17, 19, and 24 hour days. The control plant was grown in a greenhouse. The photograph shows the extreme injury of continuous illumination on tomato plants.

through the increase or decrease of nitrate in the soil providing the plant has no regulatory mechanism which limits the absorption of this salt. It should also be possible to initiate or inhibit flowering by adjusting light intensity in combination with suitable day length and in this way regulate the ratio through an increase or decrease of carbohydrate, providing again that the plant has no regulatory mechanism for maintaining only a certain amount of carbohydrate reserve. In previous work it has been observed that certain plants such as corn are able to regulate the amount of nitrate taken in so that it is difficult to induce this plant to take up enough nitrate to increase the total percentage of nitrogen in the plant. The most promising method of changing the carbohydrate-nitrogen balance therefore seemed to be an increase of light intensity in combination with suitable day lengths. Both the decrease and increase of nitrate in the soil as well as different light intensities in combination with various day lengths have been used in this study. The analytical data are reported in tables 4 to 9.

In the case of long and short day plants the tables are divided into two parts, those which either were flowering or had the flowering response, and those which were not flowering or had no flowering response. Flowering response is here used to mean the ability of a long day plant to flower after it has been kept for a time on a long day and then transferred to a short day where it will later flower. This is a common characteristic of both radishes and lettuce. The opposite situation also exists; that of a short day plant flowering on a long day after it has been transferred, but this has not been studied carefully in the work reported herewith.

The "dose" of light necessary to initiate flowering in long-day plants growing in greenhouses during the short days of winter involved mainly the length of day (photographs of long and short day and "everblooming" types are shown in text figs. 5, 6 and 7). Intensity was not a factor since the lowest intensity used (170 foot candles) was well above the minimum for initiating flowering. While this exact minimum has not been accurately determined it is known to be very low as compared with sunlight. Light diffusing from the gantry crane greenhouse in these experiments induced flowering in radish and lettuce in another greenhouse at an illumination value of about five foot candles. Intensities of this order produce little or no weight increase in plants, and are probably well below the minimum for survival.

The total carbohydrate and total nitrogen in percent of dry weight for radish and lettuce together with the ratio of the two are listed in tables 4 and 5. The great variation of these fractions in plants grown under various conditions is shown. Total carbohydrate in radish (table 4) varies from 7.47 to 34.73 percent among the plants which were flowering or showed flowering response, while there was a similar range, 8.95 to 21.23 percent, among those which did not flower. Total nitrogen varied from 1.51 to 5.92 percent in the first case and from 2.77 to 7.27 percent in the second.

TABLE 4. *Carbohydrate-nitrogen Relation in Radish, a Long Day Plant. Plants Grown With Various Lengths of Day. Whole Aërial Portion*

| Treatment of Plant, Age, and Number of Days in Growth Condition | Total Carbo- hydrate, % Dry Weight | Total Ni- trogen, % Dry Weight | Carbohydrate Nitrogen |
|--|--|--------------------------------------|--------------------------|
| 1. Plants flowering or showing flowering response when transferred to short day. 1927 Series unless otherwise indicated | | | |
| Control greenhouse, 51 days (1), F..... | 9.67 | 4.91 | 2.0 |
| Greenhouse 2, 21 days, F.S.D..... | 13.14 | 5.75 | 2.6 |
| “ “ 2, 41 days, F..... | 19.94 | 3.77 | 5.3 |
| 15 hour day, 52 days, F..... | 15.41 | 3.36 | 4.6 |
| 17 “ “ 45 days, F..... | 9.59 | 2.45 | 4.1 |
| 19 “ “ 20 days, F.S.D..... | 21.90 | 4.67 | 4.7 |
| 19 “ “ 40 days, F..... | 21.90 | 2.87 | 7.6 |
| 24 “ “ 29 days, F.S.D..... | 25.47 | 4.68 | 5.4 |
| 24 “ “ 35 days, F..... | 27.53 | 3.45 | 8.0 |
| 24 “ “ 31 days, F..... | 17.15 | 4.00 | 4.3 |
| 24 “ “ 24-hour night, 14 days, F..... | 10.19 | 5.63 | 1.9 |
| Greenhouse + 8 hours artificial light 170 f.c. 15 days, F.S.D. (2)..... | 7.47 | 5.92 | 1.3 |
| Greenhouse + 8 hours artificial light 170 f.c. 44 days, F. (2)..... | 7.87 | 5.72 | 1.4 |
| Greenhouse 1, 46 days, 1926 F.—grown in sand | 27.44 | 1.51 | 18.1 |
| Greenhouse 2, 46 days, 1926 F.—grown in sand | 34.73 | 1.52 | 22.8 |
| 2. Plants not flowering and having no flowering response | | | |
| Greenhouse control, 32 days (4)..... | 12.69 | 5.99 | 2.1 |
| “ “ “ 51 days..... | 18.50 | 7.27 | 2.6 |
| 7 hour day—40 days..... | 10.55 | 5.88 | 1.8 |
| 7 “ “ 54 “..... | 8.95 | 6.33 | 1.4 |
| 12 “ “ 20 “..... | 19.14 | 5.70 | 3.4 |
| 12 “ “ 61 “..... | 8.89 | 5.23 | 1.7 |
| 15 “ “ 20 “ (3)..... | 17.67 | 4.69 | 3.8 |
| 17 “ “ 20 “ (3)..... | 21.12 | 4.84 | 4.4 |
| Greenhouse + 8 hours artificial light 170 f.c. 7 days (age 31 days)..... | 12.34 | 5.98 | 2.1 |
| Control greenhouse—31 days..... | 10.70 | 5.72 | 1.9 |
| 12 hour day—12 hour night—14 days..... | 10.16 | 5.22 | 2.0 |
| Greenhouse control + .3 percent CO ₂ —7 days (age 31)..... | 14.43 | 5.35 | 2.7 |
| Greenhouse control + .3 percent CO ₂ —15 days (age 39)..... | 12.88 | 5.58 | 2.3 |
| Greenhouse control + .3 percent CO ₂ —20 days (age 44)..... | 11.43 | 4.88 | 2.4 |
| Greenhouse control grown in sand 46 days. 1926..... | 21.23 | 2.77 | 7.7 |
| Greenhouse control grown in soil 46 days. 1926..... | 13.93 | 4.87 | 2.9 |

F. With flower stalks.

F.S.D. The same series flowered later when transferred to a short day.

(1). These plants flowered in a house adjacent to gantry crane house due to the entrance of diffused light of about 5 f.c.

(2). Some of these plants were sampled after 15 days and 3 out of 12 of this series flowered when returned to a short day. When sampled after 44 days flower stalks were appearing.

(3). None of the plants of this series flowered when returned to a short day.

(4). Sampled Feb. 28, 1927.

Note: Greenhouse 2, 1927 received daylight plus 12 hours of artificial light each night from the gantry crane. Carbon dioxid concentration about .3 percent. Temperature 78° F. 7 to 24 hour day plants grown in constant-light room with CO₂ concentration and temperature same as greenhouse 2. Greenhouses 1 and 2 in 1926 received 6 hours of light each night from the gantry crane. CO₂ concentration increased in 2. Temperature 68° F.

TABLE 4A. *Analysis of Radish Tops, Showing the Relation of Carbohydrate and Nitrogen Fractions to Length of Day. Constant-light Room*

| Day Length | Wet Weight per Plant | Percentage Moisture | Nitrogen Percentage | | | | Acid Hy- drolyzable Percentage | Sucrose Percentage | Dextrose Percentage | Total Carbo- hydrates Percentage | |
|-------------------|-------------------------|------------------------|---------------------|------------|-------|------|--------------------------------------|-----------------------|------------------------|--|-------|
| | | | Insoluble | Soluble | Total | | | | | Wet | Dry |
| | | | Dry Weight | Dry Weight | Wet | Dry | Dry Weight | Dry Weight | Dry Weight | | |
| 1925 | | | | | | | | | | | |
| 7-hour | 10 | 92.9 | 2.87 | 2.23 | .36 | 5.11 | 7.58 | .00 | + | .54 | 7.58 |
| 12-hour | 20 | 92.7 | 2.21 | 1.79 | .29 | 4.00 | 8.65 | .00 | 4.37 | .95 | 13.02 |
| 17-hour | 81 | 90.7 | 1.35 | .46 | .17 | 1.81 | 12.68 | + | 9.01 | 2.02 | 21.69 |
| 19-hour | 30 | 90.7 | 1.88 | 1.50 | .31 | 3.38 | 12.56 | + | 9.41 | 2.05 | 21.97 |
| 24-hour | 16 | 84.9 | 1.62 | 1.05 | .40 | 2.67 | 14.16 | + | 6.85 | 3.18 | 21.01 |
| 1926 | | | | | | | | | | | |
| 5-hour | 9.4 | 94.1 | 3.32 | 2.64 | .36 | 5.96 | 8.56 | .00 | 1.83 | .62 | 10.39 |
| 7-hour | 8.7 | 93.7 | 2.86 | 2.20 | .35 | 5.06 | 7.89 | .69 | 3.71 | .86 | 12.29 |
| 12-hour | 23.0 | 92.1 | 2.42 | .82 | .26 | 3.24 | 9.28 | .00 | 4.14 | 1.07 | 14.42 |
| 17-hour | 33.0 | 90.6 | 2.06 | .76 | .26 | 2.82 | 12.97 | .47 | 8.78 | 2.08 | 22.22 |
| 19-hour | 32.0 | 86.9 | 1.38 | .34 | .23 | 1.72 | 19.00 | 1.96 | 8.29 | 3.82 | 29.25 |
| 24-hour | 16.2 | 88.7 | 2.07 | 1.09 | .36 | 3.16 | 13.15 | 1.11 | 7.23 | 2.44 | 21.49 |
| 1927 | | | | | | | | | | | |
| 7-hour | 7.0 | 92.1 | 2.75 | 3.58 | .50 | 6.33 | 7.48 | .00 | 1.47 | 0.71 | 8.95 |
| 9-hour | 13.0 | 92.7 | 2.49 | 2.21 | .34 | 4.70 | 8.53 | .00 | 2.82 | 0.84 | 11.35 |
| 12-hour | 17.0 | 93.9 | 2.44 | 2.79 | .32 | 5.23 | 8.30 | .00 | .59 | .55 | 8.89 |
| 15-hour | 28.0 | 91.8 | 2.03 | 1.33 | .28 | 3.36 | 11.46 | .00 | 3.95 | 1.26 | 15.41 |
| 17-hour | 20.0 | 86.7 | 1.42 | 1.03 | .33 | 2.45 | | + | 2.57 | | |
| 19-hour | 5.2 | 88.4 | 1.87 | 1.00 | .34 | 2.87 | 13.52 | 1.85 | 6.53 | 2.53 | 21.90 |
| 24-hour | 8.0 | 90.8 | 2.28 | 1.72 | .37 | 4.00 | 11.26 | 1.28 | 4.61 | 1.57 | 17.15 |

+ Only a trace present.

TABLE 5. *Carbohydrate-nitrogen Relation in Lettuce, a Long-day Plant, Grown With Various Lengths of Day. Analyses of Whole Aerial Portion*

| Treatment of Plant, and Number of Days in Growth Condition | Total Carbo- hydrate, % Dry Weight | Total Ni- trogen, % Dry Weight | Carbohydrate Nitrogen |
|---|--|--------------------------------------|--------------------------|
| 1. Plants flowering or with flowering response | | | |
| Greenhouse 1—25 days—1925+ | 19.93 | 4.21 | 4.7 |
| Greenhouse 2—25 days—1925+ | 27.35 | 4.58 | 6.0 |
| 17 hour day, 25 days—1925+ | 22.01 | 3.86 | 5.7 |
| 19 " " 25 " —1925+ | 24.08 | 4.06 | 6.0 |
| 24 " " 25 " —1925+ | 26.32 | 3.78 | 7.0 |
| Greenhouse 1—60 days—1926+ grown in sand | 16.10 | 3.07 | 5.3 |
| Greenhouse 1, 60 days—1926. Soil+ | 19.88 | 4.48 | 4.4 |
| Greenhouse 2, 60 days—1926. Sand+ | 15.26 | 1.69 | 9.2 |
| Greenhouse 2, 60 days—1926. Soil+ | 17.10 | 2.50 | 6.8 |
| 17 hour day, 60 days—1926+ | 19.44 | 2.88 | 6.8 |
| 19 hour day, 60 days—1926+ | 26.92 | 3.61 | 7.4 |
| 24 hour day, 60 days—1926+ | 28.54 | 3.12 | 9.2 |
| 15 hour day, 21 days—1927— | 23.10 | 5.56 | 4.2 |
| 15 hour day, 61 days—1927+ | 21.22 | 3.24 | 6.5 |
| 17 hour day, 20 days—1927— | 23.75 | 4.50 | 5.3 |
| 17 hour day, 61 days—1927+ | 20.84 | 2.37 | 8.9 |
| 19 hour day, 21 days—1927— | 26.68 | 4.68 | 5.7 |
| 19 hour day, 61 days—1927+ | 18.12 | 2.39 | 7.6 |
| 24 hour day, 19 days—1927— | 25.75 | 4.62 | 5.6 |
| 24 hour day, 45 days—1927+ | 38.28 | 4.85 | 7.9 |
| 2. Plants not flowering and with no flowering response | | | |
| Control greenhouse, 60 days—1925 | 18.57 | 4.53 | 4.1 |
| 5 hour day, 60 days—1925 | 13.53 | 4.73 | 2.9 |
| 7 hour day, 60 days—1925 | 20.92 | 4.04 | 5.2 |
| 12 hour day, 60 days—1925 | 21.04 | 4.59 | 4.6 |
| Control greenhouse, 60 days—1926 | 22.11 | 4.75 | 4.7 |
| 7 hour day, 60 days—1926 | 18.30 | 4.89 | 3.7 |
| 12 hour day, 60 days—1926 | 17.46 | 4.14 | 4.2 |
| Control greenhouse, 34 days—1927 | 22.86 | 4.95 | 4.6 |
| 5 hour day, 59 days—1927 | 14.86 | 4.11 | 3.6 |
| 9 hour day, 61 days—1927 | 18.05 | 5.09 | 3.6 |
| 12 hour day, 20 days—1927 | 12.08 | 2.73 | 4.5 |
| 12 hour day, 61 days—1927 | 18.80 | 4.21 | 4.5 |

+ Flower stalk visible.

— No flower stalk visible, would flower later.

5–24 hour day grown in constant-light room with artificial light entirely and with glass-water filter. 1925 series temperature 78° F. and illumination about 970 f.c. 1926 series temperature 68° F. and illumination about 1200 f.c. 1927 series 78° F. and 1200 f.c. illumination.

There is apparently no relation between percentage composition of carbohydrate and nitrogen and flowering in the radish, since these can be varied quite independently of the flowering. Flowering can be initiated by illuminating the radish for eight hours each night with 170 foot candles with no resultant accumulation of carbohydrates. Flowering can also be initiated with a much higher intensity, 700 foot candles (Greenhouse 1,

1926), for six hours each night, with considerable accumulation of carbohydrates. When the plants were grown in sand (Greenhouse 2, 1926) there was a further accumulation in total carbohydrates to the maximum for the series at 34.73 percent. Total nitrogen was reduced to 1.52 percent. These plants flowered. Control plants grown in sand on short days did not flower but accumulated considerable carbohydrate, 21.23 percent. Total nitrogen fell to 2.77 percent in this case. Of the plants grown in the constant-light room in 1927 on 7, 12, 15, 17, 19, and 24 hour days, the plants on the four longest day lengths flowered, while those of 12 hours and less did not flower. The effect of age of the plant at the time of sampling is shown in table 6. This is also discussed later. All radish plants listed in table 4 were grown from seed which was planted in each condition, except the 12 and 24 hour day, 12 and 24 hour night plants, and those grown with daylight plus eight hours of artificial light at 170 foot candles. These plants were grown in the control greenhouse and then transferred to the various conditions. It will be observed that the 24 hour day, 24 hour night and 12 hour day, 12 hour night plants in table 2 had practically the same percentage composition of carbohydrate and nitrogen. This might be expected since these plants were grown under the same conditions except for the duration of exposure to light. The set grown on the long day flowered while those on the 12 hour day did not flower. The soluble nitrogen in percentage of dry weight was 2.44 for the 12 hour and 2.82 for the 24 hour day plants as compared with 2.78 and 2.80 for the respective insoluble fractions. There is therefore no significant difference in these fractions. Soluble nitrogen in general parallels closely the total nitrogen values. The listing of this fraction has been omitted from table 4 and several other tables to save space.

It is of especial interest to note that the highest ratio of carbohydrate to nitrogen in radish is produced by an 18 hour day (Houses 1 and 2) where nitrate supply has been limited by growing the plants in sand. In this case large amounts of carbohydrate are produced, nitrogen supply is a limiting factor to further growth, and carbohydrates accumulate. This makes no difference with flowering since the plants flowered on the long day regardless of carbohydrate-nitrogen relations. When grown in sand in the control greenhouse on the normal day length the carbohydrate-nitrogen ratio of radish plants was also very high (7.7). The value was about equal to the ratio obtained (7.6) by growing plants in soil on a 19 hour day. The control plants did not flower while those grown on the 19 hour day flowered. The values given in table 4 are averages of a large number of plants. Any number can be grown similarly which will give individuals with values in very close agreement. Flowering in radish is therefore believed to be quite independent of the carbohydrate-nitrogen relations and depends only upon day length.

In table 4A are presented chemical data showing the trend of various

carbohydrate and nitrogen fractions in radish plants as day length increases. The long day plants represented in this table were flowering when sampled. The table shows the percentage composition of insoluble and soluble nitrogen and acid hydrolyzable, sucrose and dextrose, carbohydrate fractions on a dry weight basis. Percentage total nitrogen and total carbohydrate are given on both the green weight (wet) and dry weight basis. Weight per plant and percentage moisture are also given. As pointed out above total carbohydrates and weight per plant increase with day length to 17 or 19 hours while a corresponding increase is not maintained up to a 24 hour day. Weight per plant follows a similar curve. Total, soluble, and insoluble nitrogen on a dry weight basis decrease with increasing carbohydrates. The sucrose fraction is near zero on short day lengths but increases on 17, 19, and 24 hour days. This fraction decreases with age of plants. Only one other plant studied in these experiments contained as little sucrose. This was the coleus. Both the Golden Bedder and variegated varieties of this species gave no test for sucrose when grown on either long or short days.

The carbohydrate-nitrogen relation for lettuce grown under the different conditions is summarized in table 5. The analyses of plants listed in this table shows a considerable range of carbohydrate and nitrogen percentages both in the case of the plants which flowered or had the flowering response and in the plants which did not flower and had no flowering response. The data in this table are based on the analysis of only two to ten plants in each sample and are therefore much more variable than the data for radish presented in table 3 in which large numbers of individuals were used in each sample. There is no relation between the percentage carbohydrate or nitrogen and the tendency to flower in lettuce shown by these data. Carbohydrates, in general, increase with increasing length of day in both radish and lettuce. In contrast with radish, lettuce plants did not show any increase in carbohydrate when grown in sand as compared to a good soil.

In order to determine some of the effects of age of the plants at sampling time on carbohydrate and nitrogen fractions, a series of experiments was made in 1927. Lettuce and radish plants were analyzed first when the plants were young and vigorously vegetative and later when they were flowering or beginning to flower on longer day lengths. The data from this series of analyses are given in table 6. The weight per plant of lettuce increased with day length up to a 17 hour day while there was no proportional increase on 19 and 24 hour days. The aerial portion and roots of radish follow a similar curve. The amount of water in the aerial portion in general decreases with age. The acid hydrolyzable fraction increases slightly in lettuce with age. In the aerial portion of radish there is a slight decrease, while the roots show a tendency to increase. Sucrose, dextrose and total carbohydrates in general decrease with age in both

TABLE 6. *Some Effects of Age on the Chemical Composition of Radish and Lettuce Plants. 1927 Series. Whole Aërial Portion*

| No. of Days in Condition, and Day Length | Wt. per Plant, Grams | Moisture % | Total Nitrogen % | | Acid Hydro- lyzable % | | Sucrose % | | Dextrose % | | Total Carbohy- drates % | |
|---|----------------------------|---------------|---------------------|------|--------------------------|------|--------------|------|---------------|-----|----------------------------|------|
| | | | Green | Dry | Green | Dry | Green | Dry | Green | Dry | Green | Dry |
| Lettuce, whole aerial portion | | | | | | | | | | | | |
| Control greenhouse, 34 days | 6.3 | 91.5 | .42 | 4.95 | .71 | 8.3 | .81 | 9.5 | .43 | 5.1 | 1.95 | 22.4 |
| Control greenhouse, 51 days | 10.8 | 92.8 | .33 | 4.64 | .50 | 7.0 | .42 | 5.9 | .37 | 5.1 | 1.29 | 18.0 |
| 12 hour day, 20 days..... | 6.0 | 91.8 | .23 | 2.73 | .46 | 5.5 | .28 | 3.4 | .26 | 3.2 | 1.00 | 12.1 |
| 12 hour day, 61 days..... | 45.0 | 90.4 | .24 | 2.52 | .44 | 4.6 | .35 | 3.7 | .29 | 3.1 | 1.08 | 11.3 |
| 15 hour day, 21 days..... | 13.0 | 93.7 | .35 | 5.56 | .42 | 6.8 | .57 | 9.1 | .45 | 7.2 | 1.45 | 23.1 |
| 15 hour day, 61 days..... | 64.0 | 90.4 | .31 | 3.24 | .91 | 9.4 | .73 | 7.6 | .40 | 4.2 | 2.04 | 21.2 |
| 17 hour day, 20 days..... | 20.0 | 92.8 | .33 | 4.50 | .49 | 6.8 | .71 | 9.9 | .51 | 7.1 | 1.71 | 23.8 |
| 17 hour day, 61 days..... | 135.0 | 88.8 | .27 | 2.37 | 1.23 | 10.9 | .80 | 7.1 | .32 | 2.8 | 2.35 | 20.8 |
| 19 hour day, 21 days..... | 29.0 | 93.1 | .32 | 4.68 | .56 | 8.1 | .74 | 10.7 | .54 | 7.8 | 1.84 | 26.7 |
| 19 hour day, 61 days..... | 53.0 | 86.6 | .32 | 2.39 | 1.36 | 10.2 | .70 | 5.2 | .37 | 2.8 | 2.43 | 18.1 |
| 24 hour day, 19 days..... | 23.0 | 91.9 | .37 | 4.62 | .78 | 9.6 | .78 | 9.6 | .53 | 6.5 | 2.09 | 25.8 |
| 24 hour day, 45 days..... | 80.0 | 91.7 | .40 | 4.85 | 1.31 | 15.8 | 1.18 | 14.3 | .68 | 8.1 | 3.17 | 38.3 |

TABLE 6.—*Continued*

| No. of Days in Condition, and Day Length | Wt. per Plant, Grams | c Moisture | Total Nitrogen % | | Acid Hydro- lyzable % | | Sucrose % | | Dextrose % | | Total Carbohy- drates % | |
|---|----------------------------|---------------|---------------------|------|--------------------------|------|--------------|------|---------------|------|----------------------------|------|
| | | | Green | Dry | Green | Dry | Green | Dry | Green | Dry | Green | Dry |
| Radish, whole aerial portion | | | | | | | | | | | | |
| 12 hour day, 20 days..... | 2.2 | 92.0 | .46 | 5.70 | 1.04 | 13.0 | .23 | 2.9 | .27 | 3.3 | 1.54 | 19.1 |
| 12 hour day, 53 days..... | 17.0 | 91.0 | .32 | 3.49 | 1.02 | 11.0 | + | + | .40 | 4.3 | 1.47 | 15.9 |
| 15 hour day, 20 days..... | 3.0 | 92.2 | .37 | 4.69 | 1.00 | 12.8 | .25 | 3.2 | .13 | 1.7 | 1.38 | 17.7 |
| 15 hour day, 52 days..... | 28.0 | 91.8 | .28 | 3.36 | .94 | 11.5 | + | + | .32 | 4.0 | 1.26 | 15.4 |
| 17 hour day, 20 days..... | 4.3 | 91.6 | .41 | 4.84 | 1.25 | 14.9 | .25 | 3.0 | .27 | 3.2 | 1.77 | 21.1 |
| 17 hour day, 45 days..... | 20.0 | 86.7 | .33 | 2.45 | .90 | 6.7 | + | + | .34 | 2.6 | 1.28 | 9.6 |
| 19 hour day, 20 days..... | 4.4 | 90.9 | .42 | 4.67 | 1.51 | 16.6 | .13 | 1.47 | .35 | 3.8 | 1.99 | 21.9 |
| 19 hour day, 40 days..... | 5.2 | 88.4 | .34 | 2.87 | 1.56 | 13.5 | .21 | 1.85 | .76 | 6.5 | 2.53 | 21.9 |
| 24 hour day, 19 days..... | 3.7 | 89.1 | .51 | 4.68 | 1.97 | 18.1 | .32 | 3.00 | .47 | 4.4 | 2.76 | 25.5 |
| 24 hour day, 35 days..... | 8.0 | 90.8 | .37 | 4.00 | 1.03 | 11.3 | .12 | 1.28 | .42 | 4.6 | 1.57 | 17.2 |
| Radish, roots only | | | | | | | | | | | | |
| 12 hour day, 20 days..... | 1.0 | 92.2 | .25 | 3.20 | .68 | 8.7 | .20 | 2.57 | 1.88 | 24.1 | 2.76 | 35.4 |
| 12 hour day, 53 days..... | 23.0 | 93.0 | .18 | 2.60 | .65 | 9.3 | .19 | 2.72 | 1.72 | 24.6 | 2.56 | 36.7 |
| 15 hour day, 20 days..... | 1.4 | 93.1 | .20 | 2.91 | .62 | 9.0 | .10 | 1.46 | 1.79 | 26.1 | 2.51 | 36.5 |
| 15 hour day, 52 days..... | 17.0 | 93.5 | .14 | 2.16 | .78 | 12.0 | .06 | .93 | .59 | 9.1 | 1.43 | 22.1 |
| 17 hour day, 20 days..... | 4.1 | 93.6 | .19 | 2.96 | .51 | 8.0 | .15 | 2.34 | 2.06 | 32.1 | 2.72 | 42.4 |
| 17 hour day, 45 days..... | 6.4 | 93.8 | .16 | 2.59 | .57 | 9.2 | .28 | 4.54 | .18 | 2.9 | 1.03 | 16.7 |
| 19 hour day, 20 days..... | 3.0 | 92.7 | .21 | 3.10 | .68 | 9.3 | .25 | 3.41 | 2.37 | 32.3 | 3.30 | 45.0 |
| 19 hour day, 40 days..... | 12.0 | 90.5 | .19 | 2.00 | 1.49 | 15.7 | .25 | 2.64 | .91 | 9.6 | 2.65 | 27.9 |
| 24 hour day, 19 days..... | 1.2 | 89.3 | .33 | 3.07 | 1.10 | 10.3 | .42 | 3.91 | 2.90 | 27.0 | 4.42 | 41.2 |
| 24 hour day, 35 days..... | 5.9 | 89.7 | .28 | 3.79 | 1.49 | 14.5 | .24 | 2.33 | 1.20 | 11.7 | 2.93 | 32.5 |

lettuce and radish. It is therefore important for purposes of comparison to choose plants of these two species which are approximately the same age.

The analytical data for salvia, a short day plant, are presented in table 7. This plant flowers well up to and including day length of 15 hours, rarely on a 17 hour day. A photograph showing the flowering on short day lengths is included as text figure 6. The carbohydrate and nitrogen fractions show a very narrow range over all day lengths from five hours to 24. On the shortest day length, five hours, in 1926, carbohydrates were high at 22.87 percent as compared with 28.19 percent on the 19 hour day, while nitrogen showed a correspondingly small variation from 3.24 percent to 2.04 percent on the same range of day lengths. The application of large amounts of nitrate to the soil made practically no difference in the total nitrogen content, although the plants showed considerable foliar injury due to the toxicity of high concentrations of this salt. The seven hour day plant receiving extra nitrate had a total nitrogen percentage of 3.35 as compared to the value 2.8 for the seven hour with no extra nitrate. The carbohydrate percentages were 14.08 and 25.45, respectively. The slight increase in nitrogen may as well be attributed to the falling off of carbohydrate due to slight foliar injury as to the increase in nitrate supply in the soil. The 17 and 19 hour day plants receiving nitrate were also slightly higher in total nitrogen and slightly lower in total carbohydrate, but compared with plants growing on short day lengths total nitrogen in those plants receiving large quantities of nitrate is very low. Salvia is able to regulate closely the total percentage composition of nitrogen in the tissues when grown in a medium with high nitrogen supply. The numerical value of this percentage depends mainly upon length of day and intensity of light. This regulatory action apparently is not restricted to salvia since various workers have found a similar relation in other species. Walster (20) found that when barley was grown at a high temperature with a high nitrogen supply in a sand medium the plants were very weak and prostrate in growth habit, whereas when grown similarly except in a cool house the plants were erect and sturdy. He found no greater differences in total nitrogen percentages, however, than may be easily attributed to variations in sampling. Hooker and Bradford (7, 8) have analyzed both the bearing fruit spurs and bark of apple twigs which have been fertilized with nitrate, ammonium sulfate, and blood. As compared with the control plants with no fertilizer they found no appreciable differences in total nitrogen percentage. The interesting thing about these observations is that the effects of high nitrogen supply are brought about in these species with no corresponding increase in percentage total nitrogen in the plants. Woo (22) found that high nitrate content of soil produced no corresponding increase of nitrogen in amaranthus plants. Tincker (19) found that the crude protein ($N \times 6.25$) in the leaves of cocksfoot grass plants could be about doubled when the plants were fed sodium nitrate at the rate of five

TABLE 7. *Carbohydrate-nitrogen Relation in Salvia, a Short-day Plant. Analyses of Whole Aërial Portion Unless Otherwise Stated*

| Treatment of Plant, Age and Number of Days in Growth Condition | Total Carbo- hydrate, % Dry Weight | Total Ni- trogen, % Dry Weight | Carbohydrate Nitrogen |
|--|--|--------------------------------------|--------------------------|
| 1. Plants flowering or with flowering response | | | |
| Control greenhouse, 62 days—1926..... | 18.47 | 3.40 | 5.5 |
| 5 hour day, 62 days—1926..... | 22.87 | 3.24 | 7.1 |
| 7 hour day, 62 days—1926..... | 25.45 | 2.80 | 9.1 |
| 12 hour day, 62 days—1926..... | 27.63 | 3.16 | 8.8 |
| 7 hour day, NaNO ₃ , 62 days, 1926 (1)..... | 14.08 | 3.35 | 4.2 |
| 12 hour day, 35 days—1927 (2)..... | 31.62 | 3.84 | 8.2 |
| 17 hour day, 35 days—1927 (2)..... | 33.56 | 3.52 | 9.6 |
| 5 hour day, 61 days—1927..... | 23.88 | 4.39 | 5.5 |
| 7 hour day, 61 days—1927..... | 23.86 | 3.99 | 6.0 |
| 9 hour day, 61 days—1927..... | 25.39 | 3.71 | 6.8 |
| 12 hour day, 61 days—1927..... | 30.59 | 2.49 | 12.3 |
| 15 hour day, 61 days—1927..... | 30.45 | 2.47 | 12.3 |
| 17 hour day, 61 days—1927..... | 30.74 | 2.84 | 10.8 |
| 2. Plants not flowering and with no flowering response | | | |
| Greenhouse 1—62 days—1926..... | 14.48 | 3.51 | 4.1 |
| Greenhouse 2—62 days—1926..... | 20.52 | 2.70 | 7.6 |
| 17 hour day, 62 days—1926..... | 25.41 | 1.93 | 13.2 |
| 19 hour day, 62 days—1926..... | 28.19 | 2.03 | 13.8 |
| 24 hour day, 62 days—1926..... | 26.18 | 2.26 | 11.6 |
| 17 hour day, NaNO ₃ , 62 days—1926 (1)..... | 23.62 | 2.34 | 10.1 |
| 19 hour day, NaNO ₃ , 62 days—1926 (1)..... | 17.64 | 2.80 | 6.3 |
| 24 hour day, 35 days—1927..... | 37.95 | 3.03 | 12.4 |
| 19 hour day, 61 days—1927..... | 31.50 | 2.56 | 12.3 |
| 24 hour day, 61 days—1927..... | 35.14 | 3.08 | 11.4 |
| Greenhouse 2—61 days—1927..... | 25.33 | 2.97 | 8.5 |
| Greenhouse + 6 hours light each night, Jan. 28—Oct. 4, 1927. Leaves only (3)..... | 22.12 | 2.66 | 8.4 |
| Same except stems only (3)..... | 21.27 | .57 | 37.3 |
| Same except from Jan. 28—Dec. 19, 1927. Leaves only (3)..... | 22.18 | 2.53 | 8.4 |
| Greenhouse control sampled Oct. 4, 1927. Leaves only..... | 16.81 | 3.31 | 5.1 |
| Same except stems only..... | 21.66 | .79 | 27.3 |

(1). Given 100 cc. of NaNO₃ solution containing 5 grams NaNO₃ at one or two week intervals in four separate doses.

(2). Other 12 and 17 hour day plants of this series were flowering when sampled after 62 days. The 17 hour day plants flowered only at the terminal while the 12 hour day plants flowered at both terminals and laterals.

(3). Received 6 hours artificial light each night from one 1000-watt lamp.

Note: 5 to 24 hour day plants grown in constant-light room. In 1926 the temperature was 68° F. and illumination about 1200 f.c. In 1927 the temperature was 78° F. and illumination about 1200 f.c. Greenhouses 1 and 2 in 1926 received 6 hours of light each night from the gantry crane. CO₂ concentration was increased in 2. Temperature 68° F.

grams weekly. This was true of plants grown either on a ten hour day or on the normal day length of June. It is evident therefore that not all plants are capable of a close regulation of total nitrogen percentage. Nightingale (16) has compared the analysis of stems from salvia plants grown on a short day of seven hours in the greenhouse during February, March, and April with those similarly grown except with full daylight supplemented at night by six hours of low intensity artificial light. The total carbohydrate in the lower stems on a percentage dry weight basis was 24.4 for the long-day plants and 23.9 for the short-day plants. The upper stems contained 19.3 and 20.0, respectively. Total nitrogen percentage of dry weight was .7 to 1.1 for the lower stems as compared with 2.0 to 1.6 for the upper from the long and short day plants. The total nitrogen percentage for the whole plant was 2.7 to 2.7. There is, therefore, no significant differences in carbohydrate or total nitrogen percentages between the long and short-day type in plants reported by Nightingale. This is probably due to the fact that the long-day plants received additional light of such a low energy value that very little additional photosynthesis took place as compared with the seven-hour day series, while the day length effects in initiating flowering are produced at a very low intensity. Nightingale found that all forms of soluble nitrogen except nitrate were relatively low in short-day salvia plants. He concludes from this that salvia is limited by a seven-hour day in the assimilation of nitrate. Since one set of plants is flowering and the other is not it would be as reasonable to conclude that other forms of soluble nitrogen were being used up by the flowers in case of the short day plants. Consequently these forms should be lower in amount in the stems. The point of especial interest is that total nitrogen and carbohydrate remain practically the same regardless of flowering.

Returning again to table 7 it will be noted that even with the narrow range of carbohydrate there is a definite increase with day length. The ability to flower is not associated with a decrease in carbohydrates since plants grown in Greenhouse 1 in 1926 with six hours of supplementary light did not flower with carbohydrate at a low value of 14.48 percent. In the 1927 series plants in one of the control greenhouses near the gantry crane house did not flower on account of diffuse light reaching these pots each night. The illumination value was less than ten foot candles. The analyses of these plants appear in table 8. The total carbohydrate percentage was 20.23. Similar plants in the control house farthest away from the crane received a much lower intensity of diffused light and did not flower. Analyses of these plants are given in table 8. The total carbohydrate percentage was 17.93. The analyses of salvia plants grown in the greenhouse with six hours of additional light each night from January until October and December are of interest. These plants were prevented from flowering during this entire period, while control plants flowered in the greenhouse in March, April, September and October. The carbohydrate

TABLE 8. *Some Effects of Day Length on the Chemical Composition of Salvia Plants. Analysis of Whole Aërial Portion*

| Conditions of Growth | Wt. per Plant, Grams | % Moisture | Nitrogen % | | | Carbohydrate % | | | | | | | |
|------------------------------------|----------------------|------------|------------|-------|------|-------------------|-------|---------|------|----------|------|-------|-------|
| | | | Soluble | Total | | Acid Hydrolyzable | | Sucrose | | Dextrose | | Total | |
| | | | | Dry | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | Wet |
| 1926 Series. 62 days in conditions | | | | | | | | | | | | | |
| Control greenhouse* | 135 | 86.1 | .74 | .47 | 3.40 | 2.01 | 14.41 | .29 | 2.10 | .27 | 1.96 | 2.57 | 18.47 |
| Greenhouse 1 | 70 | 85.7 | .68 | .51 | 3.51 | 1.68 | 11.72 | .24 | 1.70 | .15 | 1.06 | 2.07 | 11.48 |
| Greenhouse 2 | 185 | 85.7 | .48 | .39 | 2.70 | 2.39 | 16.70 | .32 | 2.32 | .21 | 1.50 | 2.93 | 20.52 |
| 5 hour day* | 93 | 87.7 | .80 | .40 | 3.24 | 2.49 | 20.24 | .12 | .97 | .20 | 1.66 | 2.81 | 22.87 |
| 7 hour day* | 126 | 84.6 | .79 | .43 | 2.80 | 3.43 | 22.24 | .27 | 1.76 | .22 | 1.45 | 3.92 | 25.45 |
| 12 hour day* | 151 | 81.3 | .47 | .59 | 3.16 | 4.51 | 24.12 | .38 | 1.96 | .29 | 1.55 | 5.18 | 27.63 |
| 17 hour day | 18 | 92.5 | .46 | .34 | 1.93 | 4.15 | 23.65 | .12 | .68 | .19 | 1.08 | 4.46 | 25.41 |
| 19 hour day | 35 | 80.7 | .40 | .40 | 2.03 | 5.11 | 26.41 | .17 | .88 | .17 | .90 | 5.45 | 28.19 |
| 24 hour day | 20 | 79.2 | .44 | .47 | 2.26 | 4.79 | 23.04 | .38 | 1.86 | .27 | 1.28 | 5.44 | 26.18 |
| 1927 Series. 61 days in conditions | | | | | | | | | | | | | |
| (1) Control greenhouse | 78 | 85.2 | .90 | .57 | 3.87 | 2.34 | 15.77 | .50 | 3.39 | .16 | 1.07 | 3.00 | 20.23 |
| Control greenhouse* | 100 | 87.0 | 1.18 | .54 | 4.20 | 1.78 | 13.67 | .29 | 2.23 | .26 | 2.03 | 2.33 | 17.93 |
| Greenhouse 2 | 341 | 87.0 | .87 | .38 | 2.97 | 2.78 | 21.29 | .36 | 2.80 | .16 | 1.24 | 3.30 | 25.33 |
| 5 hour day | 43 | 89.6 | 1.54 | .46 | 4.39 | 2.03 | 19.61 | .33 | 3.21 | .11 | 1.03 | 2.47 | 23.85 |
| 7 hour day* | 132 | 89.4 | 1.47 | .43 | 3.99 | 2.23 | 20.89 | .22 | 2.02 | .10 | .95 | 2.55 | 23.86 |
| 9 hour day* | 129 | 89.1 | 1.25 | .41 | 3.71 | 2.44 | 22.31 | .23 | 2.06 | .11 | 1.02 | 2.78 | 25.39 |
| 12 hour day* | 156 | 86.8 | .83 | .33 | 2.49 | 3.53 | 26.69 | .38 | 2.88 | .13 | 1.02 | 4.04 | 30.59 |
| 15 hour day* | 151 | 84.2 | .62 | .39 | 2.47 | 3.89 | 24.68 | .57 | 3.61 | .34 | 2.16 | 4.80 | 30.45 |
| (2) 17 hour day* | 244 | 87.7 | .81 | .35 | 2.89 | 3.28 | 26.60 | .33 | 2.61 | .19 | 1.53 | 3.80 | 30.74 |
| 19 hour day | 346 | 86.2 | .71 | .36 | 2.56 | 3.80 | 27.53 | .36 | 2.59 | .19 | 1.38 | 4.35 | 31.50 |
| 24 hour day | 179 | 84.1 | .94 | .43 | 3.08 | 4.22 | 30.58 | .45 | 3.23 | .18 | 1.33 | 4.85 | 35.14 |

* Flowering.

(1). Did not flower on account of diffused light of less than 10 foot candles from gantry crane greenhouse.

(2). Flowered on one terminal only. Greenhouses 1 and 2 in 1926 received 6 hours extra light each night from gantry crane. No. 2 also received extra CO₂. Temperature 68° F. In 1927 2 received 12 hours each night from the crane making a 24 hour day, 12 hours of which was daylight. Temperature 78° F. All other day-length plants were grown in the constant-light room.

and nitrogen fractions showed very little change at the end of this exposure, as is indicated in table 7.

The variation of various fractions of carbohydrate and nitrogen constituents of salvia plants on different day lengths are shown in table 8. The 1926 series was grown at a temperature of 68° F. as compared with 78° F. in the 1927 series. Weight per plant is maximum on a 12 hour day in the low temperature series as compared with a 19 hour day in the high temperature series. Salvia grows better at the higher temperature. The data show an increase in the weight per plant, total carbohydrates, and nitrogen in those grown at the higher temperatures as compared with the low temperature series. The increase in nitrogen appears in both the soluble and total fractions. The increase in carbohydrate is mainly in the acid hydrolyzable and sucrose fractions. Total carbohydrate increases with day length and nitrogen decreases in both the 1926 and 1927 series. An especially noteworthy characteristic of this plant is the ability to maintain a high total carbohydrate value on a five and seven hour day, resulting in a comparatively narrow range in percentage carbohydrate between a five and a 19 hour day.

The data from the analyses of buckwheat plants grown on various day lengths are especially interesting (table 9). This plant flowers on all day lengths from five to 24 hours (fig. 7). The height varies from about 18 inches on a five hour day to 52 inches on a 19 hour day. Total carbohydrates increase and total nitrogen decreases from a five to a 24 hour day. Total nitrogen usually decreases to less than one percent of the dry weight on a 24 hour day. The weight per plant increases regularly with day length up to a 19 hour day. A corresponding increase is not maintained up to continuous 24 hour illumination. The leaves of the 24 hour day plants show considerable injury as compared with the 17 hour day plants, but buckwheat in contrast to tomato, is able to continue to grow and flower on a 24 hour day. The injury from continuous light consists in the slight dying back of the leaf margins for a short distance and as the inner region of the lamina continues to grow the margin has a tendency to turn upward producing a shallow cuplike appearance. This effect can be seen in text figure 7 on both the 19 and 24 hour day plants.

Data have already been presented showing that the percentage composition of total nitrogen and total carbohydrate has little effect on flowering in the species of long and short day plants studied or in the everblooming types. In general, the percentage of carbohydrates increases with length of day where light intensity is high accompanied by a decrease in nitrogen. Flowering is initiated by a long or short day depending upon the species or variety, and is independent of the percentage composition of total easily available carbohydrates. In other species, like buckwheat, flowering is not affected by either day length or carbohydrate composition. Garner and Allard (5) have shown that day length effects are localized in each

branch of the plant. Knott (II) has shown further that the effect in cosmos is probably restricted to a few cells at the growing point of each branch. The effect of light in initiating flowering may be directly upon the protoplasm of the cells at the growing point with no resulting change

TABLE 9. *Everblooming Plant. Carbohydrate-nitrogen Relation in Buckwheat which Flowered on All Lengths of Day. Whole Aërial Portion*

| Treatment of Plant and Number of Days in Growth Conditions | Total Carbohydrate, % Dry Weight | Total Nitrogen, % Dry Weight | Carbohydrate/Nitrogen | Weight per Plant, Grams |
|--|----------------------------------|------------------------------|-----------------------|-------------------------|
| (1) 12 hour day, 23 days—1924 | 17.56 | 2.61 | 6.7 | 11.5 |
| (1) 17 hour day, 23 days—1924 | 29.90 | 1.93 | 15.4 | 33.6 |
| (1) 19 hour day, 23 days—1924 | 28.62 | 2.08 | 14.0 | 29.0 |
| (1) 24 hour day, 23 days—1924 | 35.66 | 1.68 | 21.2 | 27.4 |
| (1) 17 hour day, 65 days—1924 | 28.49 | .80 | 35.5 | 143.0 |
| (1) 24 hour day, 65 days—1924 | 28.49 | .46 | 62.0 | 104.0 |
| Control greenhouse, 40 days—1925 | 17.99 | 3.72 | 4.8 | 16.1 |
| (4) Greenhouse 1, 33 days—1925 | 17.10 | 3.16 | 5.5 | 47.3 |
| (5) Greenhouse 2, 33 days—1925 | 32.36 | .98 | 33.0 | 78.8 |
| (2) 5 hour day, 69 days—1925 | 15.84 | 3.44 | 4.6 | 3.5 |
| (2) 12 hour day, 33 days—1925 | 19.80 | 3.17 | 6.2 | 20.9 |
| (2) 17 hour day, 33 days—1925 | 38.35 | 1.42 | 27.0 | 42.6 |
| (2) 19 hour day, 33 days—1925 | 36.29 | 1.11 | 32.5 | 56.8 |
| (2) 24 hour day, 33 days—1925 | 39.41 | 1.16 | 33.8 | — |
| Control greenhouse, 58 days—1926 | 23.15 | 3.05 | 7.7 | 11.5 |
| (4) Greenhouse 1, 58 days—1926 | 27.62 | 2.25 | 12.3 | 32.7 |
| (5) Greenhouse 2, 58 days—1926 | 37.19 | 1.08 | 34.5 | 47.2 |
| (3) 5 hour day, 58 days—1926 | 25.44 | 3.39 | 7.5 | 3.2 |
| (3) 7 hour day, 58 days—1926 | 32.93 | 2.74 | 12.0 | 4.7 |
| (3) 12 hour day, 58 days—1926 | 32.10 | 2.11 | 15.2 | 12.5 |
| (3) 17 hour day, 58 days—1926 | 31.15 | 1.01 | 31.0 | 31.6 |
| (3) 19 hour day, 58 days—1926 | 33.26 | 1.15 | 28.8 | 48.5 |
| (3) 24 hour day, 58 days—1926 | 36.93 | .98 | 36.6 | 24.5 |
| Control greenhouse, 64 days—1927 | 7.94 | 3.16 | 2.5 | 7.1 |
| (6) Greenhouse 2, 64 days—1927 | 35.57 | .84 | 42.5 | 45.4 |

(1). Grown in constant-light room with artificial light entirely and with glass-water filter. Temperature 78° F. Average illumination about 450 f.c. on soil.

(2). Grown same as (1) except average illumination about 800 f.c. on soil.

(3). Grown same as (1) and (2) except temperature 68° F. and average illumination 1200 f.c.

(4). Greenhouse 1 daylight plus 6 hours from the gantry crane each night. Temperature in 1925 experiments 78° F. in 1926 68° F.

(5). Greenhouse 2 same as (4) except extra CO₂ concentration .3 percent.

(6). Greenhouse 2 in 1927 received 12 hours artificial light, otherwise same as (5).

in composition which may be detected by chemical analysis. Knott has shown that the change from the vegetative to flowering condition in spinach and cosmos is accompanied by a decrease in catalase at the terminals. Whether the decrease in catalase is a cause of flowering or is only associated with the beginning of flower production is still in doubt. It is believed,

however, that a study of enzymes or other substances present in very small amounts in the growing tips or elsewhere offers much more promise than gross carbohydrate and nitrogen fractions in various plant organs, in explaining the mechanism of light in initiating flowering and fruit production in the plant. Since some correlations have been found in a few species between tuberous root formation and the initiation of flowering, a search is in order for specific substances or stimuli accomplishing such correlation between root and tip of stem.

Carbohydrate-Nitrogen Ratio in the Tomato

Starting with the work of Kraus and Kraybill (13) considerable study has been made of the carbohydrate-nitrogen relation in the tomato plant. These authors conclude that plants grown with an abundant supply of available nitrogen and the opportunity for carbohydrate synthesis are unfruitful, high in moisture, total nitrogen, and nitrate nitrogen, and low in reducing substances, sucrose, and polysaccharids. Fruitfulness, they found, was associated neither with highest nitrates nor highest carbohydrates, but with a condition of balance between them. Plants grown with a low nitrogen supply were found to be unfruitful, low in moisture and total nitrogen, and high in carbohydrates.

In the present study the same soil mixture was used in all studies with tomato plants, except where an attempt was made to induce recovery in tomatoes injured by exposures to continuous illumination. This will be discussed later. The soil mixture contained about one-fourth manure and the usual nitrate, potash, and phosphate salts which gardeners normally use to make up a good greenhouse soil. It is believed that the plants had all the nutrient salts which they could use during their growth period. All plants were grown in two-gallon glazed stoneware jars, except those on short tests of one to two weeks. This gave the roots ample space to produce full grown plants with many fruits.

The effect of various lengths of day on the carbohydrate-nitrogen fraction of tomato plants is shown in table 10. Carbohydrates increase with day lengths up to a 17 hour day, show no further increase on a 19 hour day, and a decrease on the 24 hour day. The 24 hour day plant had in each case become almost completely defoliated before these samples were taken. Total nitrogen decreases steadily with increasing day length up to a 17 hour day where it reaches a minimum of about one percent of the dry weight of the plant. The carbohydrate-nitrogen ratio increases with day length up to 17 or 19 hours. The plants set fruit on all day lengths from seven to 19 hours but did not fruit on either five or 24 hour day. In contrast to the observations of Kraus and Kraybill (13) it is seen that, under the above conditions, the ratio of carbohydrate to nitrogen has little to do with fruiting in the tomato. Fruiting is here taken to mean the setting and continued growth of three or four fruits per plant. High

TABLE 10. *Carbohydrate-nitrogen Relation in Tomato Plants Grown With Various Lengths of Day. Analyses of Whole Plants Except Roots and Fruits*

| Treatment of Plant and Number of Days in Growth Conditions | Total Carbo- hydrate, % Dry Weight | Total Ni- trogen, % Dry Weight | Carbohydrate Nitrogen | Weight per Plant, Grams |
|--|--|--------------------------------------|--------------------------|-------------------------------|
| Control greenhouse, 62 days— 1924 F..... | 19.14 | 2.35 | 8.2 | 556 |
| 5 hour day, 62 days—1924..... | 14.88 | 3.30 | 4.5 | 142 |
| 7 hour day, 62 days—1924 F..... | 19.07 | 2.52 | 7.5 | 312 |
| 12 hour day, 62 days—1924 F..... | 25.43 | 2.22 | 11.4 | 528 |
| 17 hour day, 62 days—1924 F..... | 31.83 | 1.85 | 17.2 | 723 |
| 19 hour day, 62 days—1924 F..... | 33.84 | 1.77 | 19.1 | 634 |
| 24 hour day, 62 days—1924..... | 20.45 | 2.82 | 7.3 | 139 |
| Control greenhouse, 40 days— 1925 F..... | 20.53 | 2.46 | 8.3 | 645 |
| 5 hour day, 70 days—1925..... | 19.95 | 4.31 | 4.6 | 41 |
| 7 hour day, 70 days—1925 F..... | 14.10 | 3.45 | 4.1 | 444 |
| 12 hour day, 70 days—1925 F..... | 33.00 | 1.09 | 30.0 | 602 |
| 17 hour day, 70 days—1925 F..... | 34.94 | 1.16 | 30.0 | 569 |
| 19 hour day, 70 days—1925 F..... | 31.94 | 1.45 | 22.0 | 487 |
| 24 hour day, 70 days—1925..... | 1.24 | 3.74 | — | 11 |
| Greenhouse 1, 70 days—1925 F..... | 25.75 | 1.34 | 19.2 | 780 |
| Greenhouse 2, 70 days—1925 F..... | 34.02 | 1.35 | 25.1 | 1433 |
| Greenhouse 2, 34 days—1926..... | 29.61 | 4.34 | 6.8 | 70 |
| Greenhouse 1, 63 days—1926 F..... | 21.82 | 1.88 | 11.5 | 512 |
| Greenhouse 2, 63 days—1926 F..... | 37.23 | .96 | 39.0 | 471 |
| 12 hour day, 34 days—1926..... | 29.84 | 3.56 | 8.4 | 86 |
| 17 hour day, 34 days—1926..... | 37.17 | 2.29 | 16.3 | 149 |
| 19 hour day, 34 days—1926..... | 17.69 | 3.29 | 5.4 | 147 |
| 5 hour day, 63 days—1926..... | 9.84 | 5.29 | 1.9 | 20 |
| 7 hour day, 63 days—1926..... | 17.15 | 3.83 | 4.5 | 43 |
| 12 hour day, 63 days—1926 F..... | 25.45 | 1.27 | 20.0 | 261 |
| 17 hour day, 63 days—1926 F..... | 43.64 | .96 | 45.5 | 278 |
| 19 hour day, 63 days—1926 F..... | 34.61 | 1.09 | 31.7 | 215 |
| 24 hour day, 63 days—1926..... | 11.33 | 4.35 | 2.6 | 29 |

F. Fruiting.

5 to 24 hour day plants grown in constant-light room with artificial light entirely and with glass-water filter. 1924 series temperature 78° F. and illumination about 450 f.c. 1925 series same temperature but illumination about 800 f.c. 1926 series grown at 68° F. and illumination about 1200 f.c.

Greenhouses 1 and 2 received 6 hours illumination each night from the gantry crane. Temperature for respective years same as 5 to 24 hour day plants. Greenhouse 2 also received extra CO₂ at about .3 percent concentration.

carbohydrate accumulation results in a rapid depletion of nitrogen fractions in tomato. This agrees with the observations of Kraus and Kraybill. High carbohydrate accumulation does not, however, result in a condition of unfruitfulness where soil nitrogen is available. No study has been made in these experiments of the ability of the tomato plant to absorb nitrate nitrogen. It is possible that this plant may absorb sufficient nitrate to produce unfruitfulness under ordinary greenhouse conditions. It is also possible that when grown in sand with limited nitrate supply a condition

of unfruitfulness may be produced. This condition might also be produced by growing plants with a shortage of other mineral nutrients. The weight per plant increased with day length up to a 17 hour day in the case of those plants grown in the constant light room in the 1924 series. The weight of the 19 hour day plants decreased slightly. In the 1925 series with intensity almost twice as high the injury was greater on the long days of 17, 19 and 24 hours so that the greatest weight was produced on a 12 hour day. As the intensity increases the length of day for maximum growth decreases in case of the tomato plant. At the low intensity of 1924 even the 24 hour day plant produced considerable increase in weight before it had lost much of its leaf tissue. The weight per plant produced in the 1926 series is not comparable since this series was grown at a lower temperature (68° F.) which is not favorable to tomato. The weight of tissue produced at all day lengths at the low temperature is much lower than either the low intensity series of 1924 or the high intensity series of 1925. The 1926 series was also grown at a higher light intensity, 1200 foot candles. This operates to cause a decrease in weight produced at all of the longer day lengths. The amount of injury therefore depends both upon intensity and day length. The greatest weight produced during these experiments was that in Greenhouse 2 in 1925 of 1433 grams per plant in 70 days. These plants were grown with daylight supplemented by six hours each night from the crane with about ten times the normal carbon dioxide concentration. This combination of light produced very little if any leaf injury. Each plant produced from six to ten large fruits some of which were ripening when sampled. The ratio of total carbohydrate percentage to total nitrogen percentage was 25.1.

In the first experiments at low light intensity in 1924 it was evident that tomato plants would not withstand continuous illumination. After the plants had been in the condition 20 days only a few small leaflets at the terminals remained alive. Those plants grown on a 19 hour day in this series retained practically all of the leaves produced, all remained green and presented a normal appearance although they grew tall due to the low intensity. With higher intensities in 1925 and 1926 the 24 hour day plants showed the first signs of injury in about five days and after four weeks not a green leaf was left on any of the plants. The 19 hour day plants developed the injury more slowly and managed to maintain several green leaves all during the experiment but the older leaf tissue was injured severely. The 17 hour day plants at the higher intensity also appeared to be slightly injured, and from the data on weight per plant in table 10 it is evident that both the 17 and 19 hour day plants were injured.

Of the plants grown in these experiments the tomato is the most sensitive to high light intensity in combination with a long day. Many plants will withstand continuous illumination with little apparent injury, others are much more susceptible. These will be discussed later.



TEXT FIG. 9. A close-up photograph of a leaf from the tomato plant in FIG. 10, showing the necrotic areas developing along the veins. TEXT FIG. 10. A tomato plant kept under continuous illumination (24 hour day) in the constant-light room in 1927 for six days. The lower leaves are yellowing while the younger leaves around the terminal have a tendency to curl back toward the main stem. TEXT FIG. 11. Tomato plants grown in the constant-light room on 17 and 24 hour days, in the control greenhouse (House 1) and in the gantry crane greenhouse on a combination of daylight supplemented by 12 hours artificial light each night (House 2). Both the 24 hour day and House 2 plants have many yellowing leaves due to continuous illumination. In conditions 20 days. TEXT FIG. 12. Cabbage plants grown in the constant-light room on 5, 7, and 17 hour days in 1925 showing the buckling and splitting of leaves on the long days when grown under a fixed light source. The 5 hour day leaves tend to remain flat.

Further Experiments on the Injury to the Tomato Plant of Continuous Illumination

Further experiments were made in 1926, 1927, and 1928 to determine a possible mechanism of injury of long days on the tomato plant. It was found that the first signs of injury appeared in five to seven days under continuous illumination. The leaves usually became faintly mottled with necrotic areas developing along the veins. Text figure 9 illustrates such a leaf from a plant under continuous illumination for six days. The leaves turn downward and backward toward the stem and slowly die back until after two or three weeks of exposure only a few small terminal leaves remain. Figure 10 shows the characteristic appearance of a tomato plant after six days of continuous illumination. The leaf shown in figure 9 was taken from this plant. New leaves appearing at the terminal become smaller and smaller until finally the whole plant dies if the intensity is kept sufficiently high. Since considerable amounts of carbohydrate accumulated on the long days of the 1924, 1925, and 1926 experiments it was at first thought that they accumulated in the leaves so much more rapidly than they could be translocated or used that this might account for the long day leaf injury. The chemical composition of whole plants grown for 63 days in continuous illumination in 1926 is given in table II, part 1. In part 2 of this same table is given an analysis of stems and leaves grown at the same time but sampled after only seven days of continuous exposure, when the injury was just appearing. These plants were all young and vigorously vegetative. It will be observed that in part 1, percentage total carbohydrates increase with day length up to a 17 hour day and percentage nitrogen decreases in old plants that have been kept under artificial illumination for 63 days. In part 2 the leaves of 24 hour day plants are much higher in total carbohydrates and lower in total nitrogen than control plants grown in the greenhouse. Analyses of stems and petioles gave a similar increase in carbohydrate but less in magnitude. If the injury developed as a result of an accumulation of carbohydrates it should be feasible to choose a light intensity which would not permit of any accumulation and in this way protect the plants. Plants were therefore grown in the light room on different intensities, using two 1500-watt lamps without filters and placing the plants at various distances from the lamps. Analyses of plants so grown at various intensities are given in table II, part 2. All of the plants in this table showed the typical long day injury except the controls grown in the greenhouse with daylight only. It will be seen that at an illumination of 150 foot candles carbohydrates do not accumulate in the leaves and that the total carbohydrate is less than in those grown in the control greenhouse during March, yet the injury develops, although more slowly. At 400 and 700 foot candles the injury develops at the usual rate so that after about three weeks' exposure the plant is almost completely defoliated. Total carbohydrates at 400 foot candles are at a

TABLE II. *Percentage Composition of Tomatoes as Affected by Day-length and Other Conditions*
I. After being kept under artificial light for 63 days. 1926. Entire plant except roots

| Growth Conditions | Moisture % | Nitrogen | | | | Carbohydrates, % Wet and Dry Weights | | | | | | | |
|-------------------|---------------|----------|-----|-------|-----|--------------------------------------|------|---------|-----|----------|-----|--------------------|------|
| | | Soluble | | Total | | Acid Hydrolyzable | | Sucrose | | Dextrose | | Total Carbohydrate | |
| | | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry |
| 5 hour day..... | 95.7 | .14 | 3.2 | .23 | 5.3 | .43 | 9.8 | + | + | + | + | .43 | 9.8 |
| 7 hour day..... | 91.4 | .14 | 1.6 | .33 | 3.8 | 1.24 | 14.3 | .11 | 1.2 | .14 | 1.6 | 1.49 | 17.2 |
| 12 hour day..... | 88.1 | .11 | .9 | .27 | 1.3 | 2.44 | 20.4 | .20 | 1.7 | .40 | 3.3 | 3.04 | 25.5 |
| 17 hour day..... | 86.8 | .02 | .2 | .13 | 1.0 | 4.49 | 33.9 | .40 | 3.0 | .89 | 6.7 | 5.78 | 43.6 |
| 19 hour day..... | 86.1 | .02 | .2 | .15 | 1.1 | 4.01 | 28.9 | .22 | 1.6 | .58 | 4.2 | 4.81 | 34.6 |
| 24 hour day..... | 91.8 | .21 | 2.5 | .36 | 4.4 | .81 | 9.9 | .04 | .5 | .08 | 1.0 | .93 | 11.3 |

TABLE II.—Continued

2. Young tomato plants grown in greenhouses and then exposed to continuous illumination for 5 to 7 days.

All of these plants showed typical long day injury except controls grown in greenhouse

A. Analysis of leaves only

| Growth Conditions and Sampling Date of Control Plants | Wt. per Plant, Grams | Moisture % | Nitrogen % Dry Weight | | Carbohydrates, % Wet and Dry Weights | | | | | | | |
|--|----------------------------|---------------|--------------------------|-------|--------------------------------------|-------|---------|------|----------|------|-------------------------|-------|
| | | | | | Acid Hydro-lyzable | | Sucrose | | Dextrose | | Total Carbohy- drate | |
| | | | Soluble | Total | Wet | Dry | Wet | Dry | Wet | Dry | Wet | Dry |
| Control 3/28/1928..... | 33 | 87.7 | .38 | 3.73 | 2.23 | 18.16 | .24 | 1.95 | .44 | 3.57 | 2.91 | 23.68 |
| Control 4/16/1928..... | 21 | 86.2 | .28 | 3.10 | 3.46 | 24.94 | .12 | .83 | .44 | 3.16 | 4.02 | 28.93 |
| Control 5/5/1928..... | 15 | 86.3 | .29 | 3.19 | 3.28 | 23.89 | .20 | 1.49 | .27 | 2.00 | 3.75 | 27.38 |
| Same, except sampled after 17 hours in dark room | 16 | 86.7 | .30 | 3.58 | 2.32 | 17.45 | .04 | .26 | .20 | 1.48 | 2.56 | 19.19 |
| 24 hour day, 700 f.c. (1)..... | 15 | 85.7 | .29 | 2.78 | 4.00 | 27.95 | .16 | 1.11 | .32 | 2.25 | 4.48 | 31.31 |
| Same, but sampled after 40 hours in dark room (1)..... | 16 | 88.9 | .58 | 3.85 | 1.06 | 9.49 | 0 | 0 | .13 | 1.20 | 1.19 | 10.69 |
| 24 hour day, 150 f.c. (1)..... | 23 | 90.8 | .76 | 4.10 | .74 | 8.00 | .01 | .10 | .11 | 1.20 | .86 | 9.30 |
| 24 hour day, 400 f.c. (1)..... | 27 | 89.5 | .37 | 3.60 | 1.71 | 16.20 | .08 | .80 | .17 | 1.60 | 1.96 | 18.50 |
| 24 hour day, 1200 f.c. (2)..... | 22 | 74.4 | .22 | 2.10 | 11.87 | 46.30 | .13 | .51 | .89 | 3.50 | 12.89 | 50.30 |
| Same, except plants 10 days old (2)..... | 46 | 75.2 | .19 | 1.50 | 11.37 | 45.90 | .29 | 1.17 | 1.38 | 5.60 | 13.04 | 52.60 |
| Greenhouse 2—2/7/1927 12 hrs. sunlight + 12 hrs. artificial | 18 | 75.5 | .20 | 1.80 | 13.38 | 54.60 | .24 | 1.00 | .35 | 1.40 | 14.00 | 57.00 |

B. Stems and petioles only

| | | | | | | | | | | | | |
|----------------------------|----|------|------|------|------|-------|-----|------|------|------|------|-------|
| Control 4/16/1928..... | 23 | 91.4 | — | — | 1.19 | 13.85 | .24 | 2.84 | .54 | 6.24 | 1.97 | 22.93 |
| 24 hour day, 150 f.c..... | 37 | 92.7 | 1.34 | 2.45 | 0.79 | 10.78 | .10 | 1.41 | .31 | 4.24 | 1.20 | 16.43 |
| 24 hour day, 700 f.c..... | 34 | 91.7 | 0.73 | 1.71 | 0.92 | 11.14 | .16 | 1.99 | .71 | 8.64 | 1.79 | 21.77 |
| 24 hour day, 1300 f.c..... | — | 89.1 | 1.04 | 1.84 | 1.56 | 14.35 | .20 | 1.83 | 1.06 | 9.76 | 2.82 | 25.94 |

(1). Exposed in the constant-light room at various distances from 2 1500-watt lamps without glass-water filter. Humidity 90 percent. Temperature 80° F.

(2). Grown in 1926 experiments in constant-light room with glass-water filter.

slightly lower level than in greenhouse plants, while at 700 foot candles (without a filter) the carbohydrates are at about the same level. The analysis of plants grown with daylight 12 hours supplemented by artificial light 12 hours from the gantry crane making a 24 hour day (Greenhouse 2, 1927) are included in this table. These plants showed an injury very similar to those growing under continuous artificial illumination in the constant-light room although not as severe. Tomato plants will not withstand a 24 hour day, 12 of which is sunlight. The rate of development of the injury, however, is decreased considerably by 12 hours of daylight in the combination. This is shown in text figure 11. The 24 hour all artificial light plant is more severely injured than the plant from Greenhouse 2. Both have been illuminated continuously since January 28, but the plant marked "House 2" received 12 hours of sunlight each day. Both plants have yellow leaves. The picture was taken on February 19, after the plants had been in the conditions 22 days. In table 10 it will be seen that total carbohydrates represent over 50 percent of the dry weight of leaves grown either on a combination of 12 hours daylight plus 12 hours artificial light, or on a 24 hour day of artificial light only at about 1300 foot candles. The total green weight of plant tissue at the end of seven days' exposure was as follows: Control greenhouse, 435 grams; 24 hour day, all artificial, 613 grams; 24 hour day, 12 of which was daylight, 497 grams. Twelve plants were used in each case. All plants grew approximately 4.5 inches in height during the experiment. It is evident, therefore, that the plants grow and increase in weight even in continuous illumination for short exposures. That they do not continue to do this is no doubt due to the breaking down of the mechanism of photosynthesis rather than to too great an accumulation of the products of this process.

To date no records have been found on the growth of tomato plants with continuous sunlight in the arctic regions. It would be interesting to know whether similar injuries develop in tomato plants grown under such natural conditions. The energy value in the constant-light room calculated at 0.3 gram calory per square centimeter per minute amounts to approximately 12,960 gram calories per month of 30 days. The total for the month of solar and sky radiation as published by the New York Observatory for June 1929 was approximately 11,903 gram calories. The two energy values are similar but as already pointed out the spectral distribution is in no way comparable. The glass-water filter in the constant-light room absorbs practically all radiation of wave length longer than 1400 $m\mu$ so that the total energy value of 12,960 gram calories includes only the visible region and the near infra-red of wave length shorter than 1400 $m\mu$.

Work already mentioned (3) has shown that sunlight has a much higher percentage of the total energy value in the visible region than the tungsten filament lamp. Since the plant uses only the energy in the visible region

and near ultra-violet for photosynthesis it is probable that the total available energy in the constant-light room per month is less than June sunlight. There is a considerable difference in the constancy of sunlight as compared to artificial light. Sunlight varies widely from minute to minute whereas the main variation in the artificial light source is brought about by the slight voltage changes of the current supply causing only insignificant fluctuations in light intensity. These differences in the two light sources can not be considered as causal agents of the injury produced by continuous artificial illumination on plant tissue since it is not known whether continuous sunlight of similar intensity will produce a similar injury.

Guthrie (6) found that chlorophyll and carotinoids decreased in the leaves of tomato plants exposed to continuous illumination. The *a* to *b* chlorophyll ratios and the carotin to xanthophyll ratios were lowered. In the case of chlorophyll, *a* decreased faster than *b*. A brown pigment, associated with a state of disturbed metabolism within the plant, increased under these conditions. It is not known whether these facts are the cause of the breaking down of the plant or only associated with it. The fact that there is a shift in the *a* to *b* ratio is especially interesting since this is normally a constant under a great range of conditions.

It is interesting to note that 12 hours of daylight in the total 24 hour light exposure per day (Greenhouse 2) decreases the severity of the injury to tomato plants but does not entirely eliminate it. During the 1927 experiments an attempt was made in the constant-light room to produce an artificial light source comparable with sunlight in spectral distribution. Three 25-ampere white flame carbon arc lamps were used in one corner of the room with 22 1500-watt incandescent lamps uniformly distributed over the rest of the ceiling of the room. The glass-water filter was used to absorb the infra-red. Tomato plants were placed on the growing benches immediately under the arc lamps. The injury developed a little more slowly in this case but the final result was the same as had been found where all incandescent filament lamps had been used. The plants died in about four weeks. White flame arc lamps furnish a better light source for growing plants on account of the quality of radiation produced, but owing to the difficulty of maintaining these lamps they are at present impractical. When the arc is not protected against rapid oxidation by a glass globe the lamp must be trimmed frequently; in the present experiments at two hour intervals. When the glass globe is used to increase the life of the carbons cerium fluorid and other metallic salts from the cores of the carbon deposit on the inner wall of the globe and produce a rapid decrease in the light output. Four mercury vapor arcs in glass tubes were also used in this study along with 25 1500-watt filament lamps to increase the blue component in the light source. Probably on account of the low energy value of the output from the mercury lamps no visible benefit was observed either on tomato plants or other plants grown in the room.

It should be noted that on account of the extreme ultra-violet radiation produced by some arc lamps these lamps should not be used for growing plants without a glass filter, unless the possibility of injury has been carefully tested. The injury of the ultra-violet region beyond the limits of sunlight has been discussed in another paper (1). The intensity and wave length of the ultra-violet produced by open arc lamps depends upon the material mixed with the carbon or the material of the core in case of cored carbons, as well as upon amperage and other factors.

Chemical Composition of Various Species of Plants Grown Under Different Conditions

Chemical Composition of Cabbage

Cabbage, variety Early Jersey Wakefield, was grown with various lengths of day in the 1924, 1925, and 1926 experiments. The chemical analysis of these plants is given in table 12. Where the plants were grown entirely with artificial light weight per plant increased with day length from five hours to 17 or 19 depending upon light intensity and temperature. Cabbage grew best at the higher temperature, 78° F., in 1925 on a medium light intensity, 970 foot candles. Plants growing on seven to 24 hour days produced heads. The best head was produced on a 17 hour day in the 1925 series. Five, seven, and 17 hour day cabbage grown with artificial light entirely are illustrated in text figure 12. All plants grown on day-lengths greater than seven hours of artificial light in this series produced warped and wrinkled leaves which later split in many places as they continued to grow. The seven hour day plant in its later stages of growth had some tendency to do this. The leaves of the five hour day plant remained perfectly smooth and flat during the experiment. As light intensity decreased the tendency to warp moved up into the longer day-length (19 and 24 hours). In the 1924 experiments plants grown at 300 foot candles without extra CO₂ produced smooth flat leaves on five, seven, 12, and 17 hour days. The mechanism of the splitting is apparently the unequal growth in different regions of the leaf lamina. It did not occur in any of the experiments where sunlight was used as part of the source of illumination. This may be due to the effect of a fixed source of light. Since cabbage leaves are almost perfectly rigid and are not able to orient themselves so as to vary the angle of incidence the rays from a fixed light source always strike parts of the leaf in the same place. This may result in a more rapid growth in certain spots of the leaf lamina, due to growth where food is most abundant. It appears also that the translocation of food in this species is mainly toward the midrib of the leaf and rarely from the center of the leaf toward the margin since otherwise the leaf margins would grow as rapidly as the rest of the leaf even if all photosynthesis took place in the center of the lamina. When illuminated with sunlight the angle of incidence is always shifting as the angle of the sun changes

TABLE 12. *Chemical Composition of Cabbage Plants*

| Treatment | Wt. per Plant, Grams | Moisture % | Nitrogen-dry Weight Percentage | | Carbohydrate-dry Weight Percentage | | | | Carbohy- drate Nitrogen |
|---|----------------------|------------|--------------------------------|-------|------------------------------------|---------|----------|-------|-------------------------------|
| | | | Soluble | Total | Acid Hydro-lyzable | Sucrose | Dextrose | Total | |
| All artificial light, constant-light room. 1924. Sampled after 70 days. Temperature 78° F. Illumination 450 foot candles | | | | | | | | | |
| Control, greenhouse leaves only . . . | 781 | 91.4 | — | 2.64 | 12.68 | 1.85 | 13.95 | 28.48 | 10.8 |
| 5 hour day, leaves only | 94 | 90.4 | — | 3.33 | 14.95 | .53 | 5.36 | 20.84 | 6.3 |
| 7 hour day, " " | 130 | 88.9 | — | 3.14 | 17.02 | 1.35 | 9.40 | 27.77 | 8.8 |
| 12 hour day, " " | 472 | 91.4 | — | 2.71 | 18.75 | 1.31 | 10.92 | 30.98 | 11.3 |
| 17 hour day, " " | 680 | 89.4 | — | 2.14 | 20.59 | 2.39 | 17.34 | 40.32 | 18.8 |
| 19 hour day, " " | 715 | 85.6 | — | 1.22 | 41.75 | 1.28 | 8.65 | 51.68 | 42.0 |
| 24 hour day, " " | 490 | 86.2 | — | 3.09 | 29.79 | 4.58 | 18.79 | 53.16 | 17.2 |
| All artificial light, except G.H. 1 and G.H. 2. 1925. Sampled after 57 days. Temperature 78° F. Illumination 900 foot candles (1) | | | | | | | | | |
| Control, greenhouse | 589 | 90.1 | .72 | 2.35 | 16.22 | 3.53 | 17.20 | 36.95 | 15.6 |
| 5 hour day | 362 | 92.5 | 1.94 | 3.36 | 12.65 | 1.42 | 11.46 | 25.53 | 7.5 |
| 7 hour day | 379 | 92.8 | 1.71 | 2.57 | 11.73 | 1.28 | 16.48 | 29.49 | 10.4 |
| 12 hour day | 476 | 92.8 | 1.26 | 2.52 | 10.24 | 1.58 | 13.09 | 24.91 | 9.9 |
| 17 hour day | 907 | 93.0 | 1.24 | 2.47 | 12.22 | 3.07 | 21.57 | 36.96 | 15.0 |
| 19 hour day | 1032 | 92.0 | 1.13 | 2.44 | 9.30 | 2.50 | 25.09 | 36.89 | 15.0 |
| Greenhouse 1 | 768 | 86.9 | .33 | 1.25 | 27.47 | 2.25 | 12.80 | 42.52 | 34.0 |
| Greenhouse 2 | 893 | 85.1 | .41 | 1.17 | 34.96 | 3.40 | 13.41 | 51.77 | 44.0 |
| All artificial light except G.H. 1 and G.H. 2. 1926. Sampled after 65 days. Temperature 68° F. Illumination 1200 foot candles (1) | | | | | | | | | |
| Control, greenhouse | 710 | 90.2 | .70 | 2.03 | 15.36 | 2.53 | 21.21 | 39.10 | 19.4 |
| 5 hour day | 248 | 91.7 | 1.56 | 3.53 | 10.41 | — | 11.23 | 21.64 | 6.1 |
| 7 hour day | 485 | 91.2 | 1.25 | 3.01 | 12.86 | — | 19.09 | 31.95 | 10.5 |
| 12 hour day | 414 | 90.2 | 1.74 | 3.30 | 8.98 | — | 16.97 | 25.95 | 7.9 |
| 17 hour day | 722 | 90.7 | 1.04 | 2.12 | 14.10 | 2.97 | 27.06 | 44.13 | 20.8 |
| 19 hour day | 633 | 86.1 | .63 | 1.43 | 26.18 | 5.22 | 23.29 | 54.69 | 38.0 |
| 24 hour day | 654 | 89.7 | .99 | 2.15 | 20.61 | 3.43 | 22.42 | 46.46 | 21.5 |
| Greenhouse 1 | 695 | 87.5 | .71 | 1.92 | 15.06 | 3.56 | 20.80 | 39.42 | 20.6 |
| Greenhouse 2 | 772 | 87.6 | .84 | 1.68 | 29.96 | 4.11 | 21.05 | 55.12 | 33.0 |

(1). Whole aerial portion analyzed.

resulting in different regions of the leaf lamina being illuminated more strongly at different times.

The percentage total carbohydrate in cabbage plants increases, and percentage total nitrogen decreases with day length from a five to a 19 hour day as shown in table 12. Percentage carbohydrates usually decreases on continuous illumination. The plant grows well on continuous illumination but reaches maximum tissue production on a day length of about 19 hours. As compared with control plants growing in the greenhouse with normal daylight during the period of the experiments both the carbohydrates and weight per plant are generally higher when normal light is supplemented by six hours of artificial light at night, making about an 18 hour day. There is a further increase in these two values in Greenhouse 2 which was given both additional light and carbon dioxid. The percentage composition of simple carbohydrates in cabbage was more than doubled on a 19 hour day as compared to a five hour day. The caloric value of such a food is greatly increased by growing it on a long day. This plant, normally considered as only a filler in an animal diet or at best a source of mineral salts and vitamins can be grown so that it has considerable fuel value as well.

Red Clover

Red clover grows exceptionally well on an 18 hour day in the gantry crane greenhouse, especially when the CO_2 concentration is increased. It was grown from seed to flower in the brief period of 38 days, in both the 1925 and 1926 series. Text figure 13 shows red clover plants flowering on April 9 in both Greenhouse 1 and 2 from seed planted February 28, 1925. Text figure 14 shows the same series on May 8 when the plants were 69 days old. A remarkably good crop of clover hay was produced in Greenhouse 2 in this brief space of time. It would take two seasons' growth to produce this in ordinary agricultural practice. The control plants did not flower during the experiment. The plants grew much better in the 1925 series at 78° . The 1926 series with a lower temperature, 68° , and a higher light intensity on the 24 hour day gave a much poorer growth. The control and Greenhouse 2 plants grown in 1927 were started a month earlier (January 28) and on account of the lower solar intensity in February did not grow as rapidly. While clover grows well even with continuous illumination the 24 hour day plant is in general no better than an 18 hour day plant. Carbohydrates and weight per plant at favorable temperatures are both increased by supplementing daylight with six hours of artificial light from the gantry crane. The chemical analyses of plants grown in 1925, 1926, and 1927 are given in table 13. Additional carbon dioxid (Greenhouse 2) produces a further increase in both weight and total carbohydrates.



TEXT FIG. 13. Red clover. The two pots of plants at the left were grown in the control greenhouse, the two center with 6 hours of artificial light supplementing daylight and with about ten times the normal concentration of carbon dioxide (gantry crane greenhouse). The two at right received the same illumination but no gas. Age from seed, 40 days. TEXT FIG. 14. Three of the same pots of clover shown in FIG. 13 with the 24 hour day plant added. The latter was grown in the constant-light room with artificial light only. Age from seed, 69 days. TEXT FIG. 15. Cucumber. Two pots at left grown in control greenhouse. Two at center in gantry crane house with daylight plus 6 hours of artificial light each night plus ten times the normal carbon dioxide. Two at right same as center except no extra gas. Plants are one month old from seed. TEXT FIG. 16. Cucumber same as FIG. 15 except 9 days later.

TABLE 13. *Chemical Composition of Red Clover. Whole Aërial Portion**

| Treatment | Wt. per Plant, Grams | Moisture % | Nitrogen-dry Weight Percentage | | Carbohydrates-dry Weight Percentage | | | | Carbohydrate Nitrogen |
|---------------------------------|----------------------|------------|--------------------------------|-------|-------------------------------------|---------|----------|-------|-----------------------|
| | | | Soluble | Total | Acid Hydrolyzable | Sucrose | Dextrose | Total | |
| Red clover, 1925. Age 76 days | | | | | | | | | |
| Control..... | 1.1 | 76.6 | .87 | 3.44 | 9.61 | 2.28 | 1.86 | 13.75 | 4.00 |
| Greenhouse 1†..... | 6.0 | 78.2 | .77 | 2.73 | 11.80 | 2.50 | 2.66 | 16.96 | 6.4 |
| Greenhouse 2†..... | 9.2 | 70.6 | .38 | 1.96 | 16.36 | 2.99 | 2.70 | 22.05 | 11.2 |
| 24 hour day†..... | 10.6 | 75.8 | .56 | 2.44 | 12.90 | 2.01 | 2.61 | 17.52 | 7.2 |
| Red clover, 1926. Age 71 days | | | | | | | | | |
| Control..... | 2.1 | 85.3 | .46 | 3.18 | 12.25 | 2.04 | 3.52 | 17.81 | 5.6 |
| Greenhouse 1†..... | 2.2 | 84.4 | .47 | 2.88 | 11.49 | 1.93 | 4.12 | 17.54 | 6.0 |
| Greenhouse 2†..... | 3.9 | 81.2 | .27 | 1.76 | 19.14 | 2.30 | 5.84 | 27.28 | 15.6 |
| 24 hour day †..... | 2.2 | 77.1 | .28 | 1.08 | 9.28 | .84 | 2.37 | 12.49 | 11.4 |
| Red clover, 1927. Age 66 days | | | | | | | | | |
| Control..... | 0.6 | 81.5 | .62 | 2.90 | 4.92 | 2.30 | 1.44 | 8.66 | 3.0 |
| Greenhouse 2 1927† (†)..... | 5.9 | 80.0 | .39 | 2.13 | 15.53 | 4.86 | 4.58 | 24.97 | 11.6 |
| 24 hour day†..... | 5.6 | 76.0 | .66 | 2.12 | 14.34 | 3.90 | 3.84 | 22.08 | 10.4 |
| 24 hour day—24-hour night†..... | 5.7 | 83.0 | .42 | 2.48 | 13.15 | 4.14 | 2.90 | 20.19 | 8.1 |

* Control plants grown in ordinary greenhouse.

Greenhouse 1, grown with daylight supplemented by 6 hours artificial light from crane.

Greenhouse 2. Same as above except extra CO₂ about .3 percent.

24 hour day, grown with artificial light only in light room with CO₂ at about .3 percent.

† Flowering.

‡ Grown with 12 hours daylight plus 12 hours of artificial light and with extra CO₂.

TABLE 14. *Chemical Composition of Soy Beans, 1925. Age 40 Days. Whole Aerial Portion**

| Treatment | Wt. per Plant, Grams | Moisture % | Nitrogen % Dry Weight | | Carbohydrate % Dry Weight | | | | Carbohydrate Nitrogen |
|--------------------|----------------------|------------|-----------------------|-------|---------------------------|---------|----------|-------|-----------------------|
| | | | Soluble | Total | Acid Hydrolyzable | Sucrose | Dextrose | Total | |
| Mandarin, control† | 7.8 | 83.0 | 1.07 | 3.71 | 19.22 | 2.06 | 1.38 | 22.7 | 6.1 |
| Mandarin, G.H. 1† | 35.8 | 80.7 | .43 | 2.97 | 19.33 | 2.00 | 2.69 | 24.0 | 8.1 |
| Mandarin, G.H. 2† | 32.1 | 77.9 | .23 | 1.81 | 26.12 | 1.81 | 3.36 | 31.3 | 17.2 |
| Peking, control† | 3.8 | 83.9 | 1.30 | 4.12 | 15.03 | 2.06 | .76 | 17.9 | 4.4 |
| Peking, G.H. 1 | 19.5 | 82.5 | .75 | 3.32 | 15.97 | 2.10 | 1.38 | 19.5 | 5.9 |
| Peking, G.H. 2 | 38.1 | 79.3 | .35 | 2.19 | 19.54 | 2.35 | 3.59 | 25.4 | 11.6 |
| Tokio, control† | 11.6 | 84.3 | 1.08 | 3.73 | 13.63 | 1.66 | .81 | 17.1 | 4.6 |
| Tokio, G.H. 1 | 30.8 | 82.5 | .70 | 3.79 | 15.22 | 2.37 | .68 | 18.3 | 4.9 |
| Tokio, G.H. 2 | 52.1 | 81.1 | .33 | 2.57 | 16.75 | 2.57 | 3.92 | 23.2 | 9.1 |
| Biloxi, control† | 14.2 | 82.4 | .93 | 3.81 | 16.40 | 1.72 | .91 | 19.0 | 5.0 |
| Biloxi, G.H. 1 | 52.8 | 80.5 | .70 | 3.19 | 14.86 | 2.54 | 1.63 | 19.0 | 6.0 |
| Biloxi, G.H. 2† | 50.8 | 75.0 | .22 | 1.50 | 30.84 | 1.82 | 2.28 | 34.9 | 23.0 |

* Grown in control or ordinary greenhouse at 78° C. Sampled 4/8/25.

Greenhouse 1, greenhouse plus 6 hours artificial light from gantry crane.

Greenhouse 2, same as above except extra CO₂.

† Fruiting or flowering.

Soy Bean

Four different varieties of soy beans were grown in the control and gantry crane greenhouses in the 1925 series, Mandarin, Peking, Tokio and Biloxi. Garner and Allard (4) observed that these varieties flowered on the following dates when grown outdoors in Washington, D. C.: Mandarin on June 15; Peking on July 10; Tokio, August 1; and Biloxi on September 1. In the present experiments Mandarin flowered and set fruit both in the control and in the greenhouses with supplementary light. Peking flowered and set fruit only in the control while Tokio did not set fruit under any of the conditions but flowered in the control greenhouse. Biloxi flowered and set a few fruit in the control and also in Greenhouse 2 with both extra light and carbon dioxid during the last few weeks of the experiment. The last observations were made on May 29, 1925, when the plants were 91 days old. It is not known whether the other varieties would have flowered in other conditions if the experiment had been continued. Since only a few plants were grown and fruiting does not occur in all individuals, the data on day length at which these varieties will flower are not conclusive. Biloxi, Mandarin and Tokio varieties grew to a height of over 40 inches during this time. The data from the chemical analysis of the four varieties are presented in table 14. It will be observed that weight per plant and total carbohydrates in general increase on the longer day in Greenhouse 1 and 2, and again in House 2 as compared with House 1. Total and soluble nitrogen and moisture decrease in the same direction.

Cucumber

Cucumber plants were grown with additional light and carbon dioxid in the 1925, 1926, and 1927 experiments. This plant was greatly favored by the higher temperatures in the 1925 and 1927 experiments. As compared to the control plants it produced more than twice the amount of tissue with additional light and gas. The increased rate of growth with additional light and gas is shown in text figures 15 and 16. The first picture was taken when the plants were one month old and the second, nine days later. The plants growing in Greenhouse 2 in 1925 attained a height of 36 inches in 30 days from the time the seed was planted. Fruits were setting in 35 days. In the last few weeks of the experiment the leaves yellowed considerably in Greenhouse 2. It was thought that this might be due to a shortage of nitrate nitrogen were carbohydrates were being built up too rapidly since leaves of the control plants remained dark green in color. In the 1926 and 1927 experiments some of the plants were given 2.5 to five grams of sodium nitrate each week for four weeks. This was effective in maintaining a dark green leaf color in all of the plants growing in the different conditions. Such high concentrations of nitrate stunted the growth of plants in the control as compared with Greenhouse 2 in which the plants were grown with extra light and carbon dioxid. The weight

TABLE 15. *Chemical Composition of Cucumber Plants. Whole Aërial Portion*

| Treatment and Sampling Date | Wt. per Plant, Grams | Moisture % | Nitrogen—Dry Weight % | | Carbohydrate—Dry Weight % | | | | Carbohydrate Nitrogen |
|--|----------------------|------------|-----------------------|-------|---------------------------|---------|----------|-------|-----------------------|
| | | | Soluble | Total | Acid Hydrolyzable | Sucrose | Dextrose | Total | |
| Greenhouse control*—4/14/1925..... | 134 | 90.4 | 1.11 | 3.95 | 15.5 | 2.45 | .99 | 18.94 | 4.8 |
| Greenhouse 1*—4/14/1925 (3)..... | 244 | 91.1 | .50 | 2.64 | 15.1 | 1.94 | 2.54 | 19.56 | 7.5 |
| Greenhouse 2*—4/14/1925 (4)..... | 315 | 89.4 | .25 | 2.01 | 26.3 | 1.66 | 3.94 | 31.88 | 15.8 |
| Greenhouse control†—4/21/1926..... | 62 | 91.4 | 1.00 | 4.38 | 15.1 | 2.04 | 1.59 | 18.74 | 4.3 |
| Greenhouse 1*—4/21/1926 (3)..... | 93 | 92.4 | 1.24 | 4.71 | 14.2 | 1.30 | 1.62 | 17.10 | 3.7 |
| Greenhouse 2*—4/21/1926 (4)..... | 210 | 88.5 | .27 | 1.82 | 25.5 | 4.33 | 4.89 | 34.74 | 19.0 |
| Greenhouse control* with NaNO_3 —4/21/26 (1)... | 60 | 87.8 | 1.35 | 3.93 | 13.8 | 2.08 | 1.94 | 17.80 | 4.6 |
| Greenhouse 2 with NaNO_3 —4/21/1926 (1) (3)... | 42 | 90.7 | 1.88 | 5.52 | 11.1 | 1.85 | 1.46 | 14.41 | 2.6 |
| Greenhouse control—3/31/1927..... | 50 | 90.7 | 1.50 | 5.35 | 13.4 | 2.57 | 1.50 | 17.45 | 3.3 |
| Greenhouse control with NaNO_3 —3/31/27 (2)... | 16 | 90.1 | 2.12 | 4.94 | 9.1 | 2.82 | .81 | 12.70 | 2.6 |
| Greenhouse 2—3/31/27—24-hour day (5)..... | 361 | 89.7 | .48 | 2.32 | 27.7 | 1.65 | 4.65 | 29.02 | 12.6 |
| Same, with NaNO_3 (5) (2)..... | 213 | 90.7 | 1.50 | 4.40 | 14.2 | 2.68 | 2.36 | 20.20 | 4.6 |

† Flowers only.

* Flowers and fruit.

(1). Received extra nitrate at rate of 5 grams NaNO_3 per week for 4 weeks.(2). Received extra nitrate at rate of 2.5 grams NaNO_3 per week for 4 weeks.

(3). Grown with 12 hours sunlight plus 6 hours of artificial light from crane making an 18 hour day. Temperature 78° F. in 1925 and 68° F. in 1926.

(4). Same as (3) except with higher concentration of CO_2 about .3 percent.

(5). Grown with 12 hours sunlight plus 12 hours of artificial light from crane making a 24 hour day. Temperature 78° F.

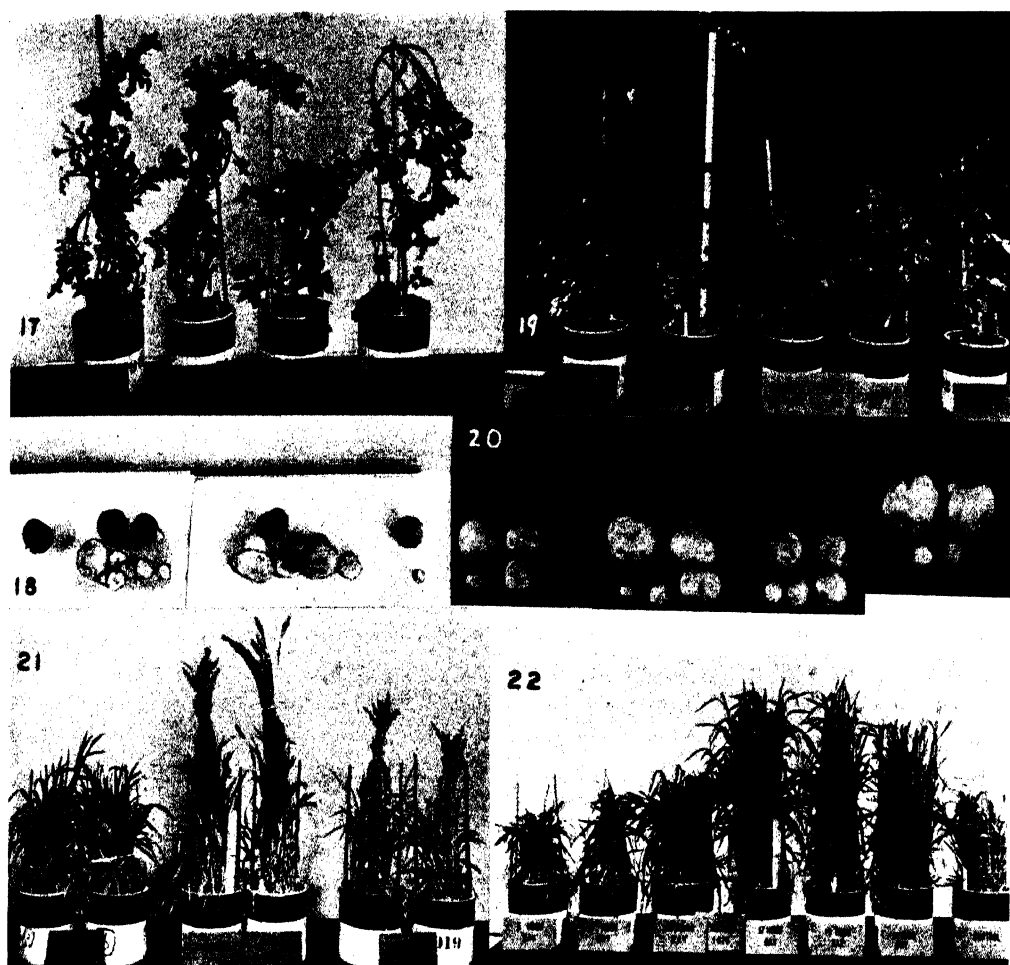
per plant as well as the chemical analysis of these plants is given in table 15. It will be observed that nitrate greatly reduced both the weight per plant and the total carbohydrate produced in most cases.

In contrast to the results with salvia, cucumber was found to absorb nitrates rapidly enough to increase the total nitrogen content considerably. This was especially true in case of the plants growing with extra light and carbon dioxid where total nitrogen was low and carbohydrate high. The controls did not absorb nitrate appreciably, but they were already high in this fraction. The application of nitrate to the soil in the case of cucumber results in a definite increase in total nitrogen where the nitrogen reserve has been depleted due to accumulation of carbohydrate material. There was no outstanding effect on flowering and fruiting where plants were fed additional nitrate except where leaf injury developed due to the toxicity of high concentration of this salt. Hand pollination was used to induce setting of fruit. Too few plants were grown in these experiments to judge relative yields accurately but plants grown with additional light and CO₂ produced larger fruit. Total carbohydrates and weight per plant increased with both additional light and with higher concentrations of carbon dioxid.

Potato

Potatoes of the Irish Cobbler variety were grown both in the gantry crane greenhouse and in the constant-light room in 1925, 1926, and 1927. The results of these experiments are of especial interest since the potato is known to be a low temperature plant. The grains, barley and spring wheat, are also commonly considered to be low temperature plants. Grains in general were found to grow well and yield well in these experiments, even at a comparatively high temperature (78° F.) if additional light and carbon dioxid is supplied. In general, many species of plants will give higher yields at a higher temperature when additional light and carbon dioxid are supplied. Tuber production in the potato in contrast with this seems limited to a low temperature. High temperature produced weak-stemmed bushy plants and little or no tuberization, although weight produced of the aërial portion at high temperature was usually greater than at low temperature. Text figures 17 and 18 show potato plants grown with a high temperature of 78° F. in 1925 together with the respective yields. Tubers marked + in text figure 18 are mother-tubers. Figures 19 and 20 show potato plants grown at a low temperature of 68° F. in 1926 and the respective yields.

Various opinions have been recorded on the effect of both day length and temperature upon tuberization of the potato. Garner and Allard (4) have given a considerable discussion of the literature on the subject and have added their own observations. They found that McCormick potatoes growing in a greenhouse during the summer did not tuberize on a long day. The temperature was comparatively very high in these experiments.



TEXT FIG. 17. Irish Clobber potato plants. Left to right, grown with daylight plus 6 hours of artificial light each night from the gantry crane, the same except extra carbon dioxide, control on normal greenhouse conditions, and 24 hour day or continuous artificial illumination in the constant-light room. Temperature 78° F. TEXT FIG. 18. Shows the yield of tubers from the plants in Fig. 17. The tubers marked + are all mother tubers. TEXT FIG. 19. Same series as FIG. 17 except grown at a lower temperature, 68° F. Arranged in different order from FIG. 17. Left to right, control, gantry crane + extra carbon dioxide (House 2), the same except no extra gas (House 1), 24 hour day all artificial light and greenhouse plus scrubbed flue gas as a source for carbon dioxide. The plants with long days and extra gas flowered especially well. TEXT FIG. 20. Shows the yield of tubers from plants grown at low temperature, FIG. 19. Long days, including continuous illumination greatly favors high yield of tubers at low temperature. TEXT FIG. 21. Barley grown at a high temperature, 78° F. The two pots of plants at left were grown in the control greenhouse, two at center in the gantry crane greenhouse with daylight plus 6 hours supplementary lighting plus a higher concentration of carbon dioxide, the two right same as center except no extra gas. TEXT FIG. 22. Barley grown at a low temperature, 68° F., in the constant-light room on 5, 7, 12, 17, 19, and 24 hour days. The plants marked control at right were grown under ordinary greenhouse conditions at the same temperature.

When grown outdoors and exposed to different day lengths, tuberization of this variety increased with day length up to a 13 hour day but fell away slightly on full day length. They conclude from these experiments that a very long day tends to direct the activities of the plant toward vegetative development. With a somewhat shorter day the tendency is toward sexual reproduction and moderate tuber formation and with further shortening of the day seed development fails and there is a tendency toward tuber formation. McClelland (15) found that weight of tops increased with day length in three varieties of potatoes. Tuberization varied with variety. Irish Cobbler produced a greater weight of tubers on a short day of ten hours as compared to a 15 hour day. Maximov (14) concluded, from his experiments, that all varieties of Russian potatoes increased tuberization with shortening of the days. Bushnell (2) found, along with many previous workers, that size of leaf and amount of tuberization decreased with increasing temperature. This, he concluded, was due to the high respiration rate at higher temperatures which used up carbohydrates too rapidly to admit of any storage in tubers.

The results of a chemical analysis of the whole aerial portions of potato plants grown in the 1926 and 1927 experiments are given in table 16. Photographs of both plants and tubers produced in the 1925 and 1926 series are shown in figures 17, 18, 19, and 20. The weight per plant of the aerial portion was greater in the high temperature series grown in 1927 (78° F.). Plants grown in the gantry crane Greenhouse 2 produced the greatest weight of top in 1927. This was an 18 hour day with additional carbon dioxide. Tuberization was very poor. The second highest in weight of tops was the 24 hour day grown in the constant-light room in 1927 at 78° F. This plant produced only one tuber about one-half inch in diameter. The greatest yields of tubers were produced by the long day plants of the cool temperature series, Greenhouse 2 and 24 hour day plants. These plants produced medium to low weight of tops. Total nitrogen was low in the 1926 low temperature series as compared with the high temperature series while carbohydrates were slightly higher. The only conclusion which can be made from these data is that this variety of potato utilizes much of the carbohydrate produced at low temperature for tuber production and very little for growth of the aerial portion. With increasing day length and high light intensity more carbohydrates are formed and consequently more and larger tubers are built up. At higher temperatures much of the carbohydrate produced is diverted toward producing growth of the aerial portion and little is available for tuber building. Increasing light intensity and day length at high temperatures results only in producing more top. Ecologically this should mean that the largest yields of potato tubers are produced in northern latitudes where air temperature is cool, day length long, and light intensity high, assuming that soil and other factors remain the same. This is generally known to be the case, high

TABLE 16. *Chemical Composition of Potato Plants. Irish Cobbler Variety*

| Treatment and Date Sampled | Wt. of Tops per Plant, Grams | Moisture % | Nitrogen, % Dry Weight | | Carbohydrate, % Dry Weight | | | | Tuber Yield |
|---|---------------------------------------|---------------|---------------------------|-------|----------------------------|---------|----------|-------|-------------|
| | | | Soluble | Total | Acid Hydro- lyzable | Sucrose | Dextrose | Total | |
| Control greenhouse—4/22/1926—68° F..... | 271 | 89.2 | .68 | 3.29 | 15.8 | 2.82 | 5.00 | 23.6 | Fair |
| Greenhouse 1—4/22/1926—68° F.*..... | 495 | 89.7 | .44 | 2.76 | 16.1 | 3.13 | 5.84 | 25.1 | Fair |
| Greenhouse 2—4/22/1926—68° F.*..... | 323 | 89.5 | .27 | 1.80 | 14.2 | 1.50 | 5.69 | 21.4 | Very good |
| 24 hour day—4/22/1926—68° F.*..... | 197 | 82.8 | .17 | .89 | 9.0 | — | 6.90 | — | Very good |
| Control greenhouse—4/1/1927—78° F..... | 246 | 92.0 | 2.13 | 5.38 | 8.3 | 2.00 | 1.50 | 11.8 | Fair |
| Greenhouse 2—4/1/1927—78° F.*..... | 862 | 89.7 | .88 | 3.41 | 13.1 | 3.31 | 2.92 | 19.3 | Poor |
| Greenhouse 1—4/1/1927—68° F..... | 307 | 90.8 | 1.75 | 5.24 | 9.6 | 3.38 | 1.31 | 14.1 | Fair |
| 24 hour day—4/1/1927—78° F.*..... | 597 | 89.1 | 1.37 | 3.75 | 12.1 | 2.74 | 2.19 | 17.0 | Poor |

* Flowering.

Greenhouses 1 and 2 in 1926 received 6 hours artificial light from crane.

Greenhouses 1 and 2 in 1927 received 12 hours artificial light from crane.

Greenhouse 2 received increased concentration of CO₂ (about .3 percent) in 1926 and 1927.24 hour day plant grown with artificial light entirely with increased CO₂.

latitudes producing much greater average yields of potatoes than equatorial, and higher altitudes greater yields than low altitudes.

Small Grains

Barley, wheat, and oats were grown in the control and gantry crane houses during most of the experiments. Barley was also grown on various day lengths in the constant-light room in the 1926 series. These grains were found to grow well even at a high temperature (78° F.) when both additional light and carbon dioxid were given. The grain yields in grams per pot of oats, barley and spring wheat (blue stem variety), were as follows:

| | Oats | Barley | Wheat |
|-------------------------|------|--------|-------|
| Control greenhouse..... | 3.46 | 4.00 | 3.78 |
| Greenhouse 1..... | 1.00 | 3.16 | 4.42 |
| Greenhouse 2..... | 2.30 | 20.50 | 9.53 |

A photograph of barley grown at a high temperature in the 1925 gantry crane series is reproduced in text figure 21. Plants grown at a low temperature in the constant-light room and control greenhouse in 1926 are shown in figure 22.

The work of Walster (20) has already been mentioned. In brief he found that barley grown at a high temperature with high nitrate supply produced weak stems and a prostrate type of growth. The prostrate habit of barley grown at 20° C. in Walster's work was no doubt produced by the low light intensity and short day conditions under which he worked. In the present experiments barley grew well and produced sturdy stems with comparatively high yields when grown continuously at 25° C. on day lengths of 17 to 19 hours (1925 series, fig. 21). When grown in the constant-light room on short days of five to 12 hours at 20° C. with an abundant supply of nitrogen a prostrate growth was produced. This is shown in text figure 22, a photograph of the 1926 series.

The results obtained from the chemical analysis of barley is tabulated in table 17 while similar data for oats and wheat are given in table 18. The weight per plant of barley increases with day length up to a 19 hour day. Total carbohydrates increase in the same way up to a 24 hour day, while total nitrogen decreases with day length. Addition of nitrate to the soil makes little or no difference in the total percentage of nitrogen in the barley plant. When total nitrogen percentage in the plant is brought to a low value by an increase in carbohydrates, due to long days and extra CO₂ concentration, the nitrogen fraction is changed very little, if any, by the addition of sodium nitrate to the soil of barley plants. This plant is apparently able to limit nitrogen intake independent of the concentration of nitrate in the soil.

In table 18 the analytical data for oats and spring wheat grown with

TABLE 17. *Chemical Composition of Barley. 30 Days Old, from Seed. 1926. Aërial Portion*

| Treatment | Wt. per Plant, Grams | Moisture % | Nitrogen, % Dry Weight | | Carbohydrate, % Dry Weight | | | | Carbohydrate Nitrogen |
|-------------------------|----------------------|------------|------------------------|-------|----------------------------|---------|----------|-------|-----------------------|
| | | | Soluble | Total | Acid Hydrolyzable | Sucrose | Dextrose | Total | |
| Control greenhouse..... | 1.2 | 89.1 | 1.62 | 5.05 | 10.52 | 3.94 | 3.06 | 17.52 | 3.4 |
| 5 hour day (1)..... | 1.2 | 91.0 | 1.89 | 5.42 | — | — | — | — | — |
| 7 hour day (1)..... | 1.9 | 90.7 | 1.84 | 5.10 | 8.06 | 3.57 | 3.13 | 14.76 | 2.9 |
| 12 hour day (1)..... | 2.8 | 90.9 | 1.87 | 4.89 | 10.60 | 3.23 | 2.66 | 16.49 | 3.4 |
| 17 hour day (1)..... | 4.5 | 86.7 | .81 | 2.93 | 16.57 | 5.58 | 7.40 | 29.55 | 10.1 |
| 19 hour day (1)..... | 4.7 | 82.9 | .44 | 1.64 | 16.22 | 8.80 | 9.49 | 34.51 | 21.0 |
| 24 hour day (1)..... | 2.9 | 84.2 | .77 | 2.70 | 17.71 | 8.55 | 10.32 | 36.58 | 13.6 |
| Greenhouse 1 (2)..... | 1.5 | 84.6 | 1.24 | 3.85 | 16.41 | 7.17 | 6.61 | 30.19 | 7.8 |
| Greenhouse 2 (3)..... | 4.3 | 83.5 | .69 | 2.69 | 15.95 | 4.87 | 8.50 | 29.32 | 10.9 |

Barley with NaNO_3 added, * 5 grams per pot per week for 2 weeks. 30 days old, from seed. 1926.

| | | | | | | | | | |
|-------------------------|-----|------|------|------|-------|-------|------|-------|--|
| Control greenhouse..... | 1.7 | 88.6 | 1.86 | 2.95 | 9.98 | 4.29 | 2.89 | 17.16 | |
| Greenhouse 1 (2)..... | 1.4 | 84.0 | 1.52 | 4.14 | 14.96 | 6.19 | 5.07 | 26.22 | |
| Greenhouse 2 (3)..... | 3.4 | 81.8 | .93 | 3.21 | 18.47 | 10.54 | 5.35 | 34.36 | |

Barley with and without extra nitrate. * Forty days old, from seed. 1927

| | | | | | | | | | |
|-------------------------|-----|------|------|------|-------|------|------|-------|------|
| Control greenhouse..... | 1.2 | 83.2 | 1.25 | 3.57 | 13.67 | 6.00 | 4.75 | 24.42 | 6.8 |
| Same—extra nitrate..... | 1.2 | 82.2 | 1.35 | 3.71 | 13.52 | 6.17 | 4.43 | 24.12 | 6.5 |
| Greenhouse 2 (3)..... | 3.5 | 79.3 | .92 | 2.51 | 19.80 | 5.55 | 1.88 | 27.23 | 10.8 |
| Same—extra nitrate..... | 4.0 | 78.2 | .78 | 2.20 | 18.14 | 8.08 | 2.16 | 28.38 | 12.8 |

* Received solution at the rate of five grams per pot of NaNO_3 each week for three weeks starting when plants were two weeks old in 1926 series. In 1927 series each pot received 2.5 grams of NaNO_3 each week for three weeks starting when the plants were three weeks old.

- (1). Grown in constant-light room with artificial light only plus extra carbon dioxide.
- (2). Grown in greenhouse with six hours supplementary light each night from crane.
- (3). Same as (2) except with extra carbon dioxide in 1926 experiments. In 1927 experiments greenhouse 2 received 12 hours supplementary light each night from crane.

TABLE 18. *Chemical Composition of Oats and Wheat. Aërial Portion*

| Treatment | Wt. per Plant, Grams | Moisture % | Nitrogen, % Dry Weight | | Carbohydrate, % Dry Weight | | | |
|---|----------------------|------------|------------------------|-------|----------------------------|---------|----------|-------|
| | | | Soluble | Total | Acid Hydrolyzable | Sucrose | Dextrose | Total |
| Oats, 1926, 51 days old | | | | | | | | |
| Control greenhouse..... | 3.5 | 84.4 | .39 | 1.80 | 16.13 | 6.86 | 3.53 | 26.52 |
| Greenhouse 1..... | 1.1 | 77.7 | .59 | 1.96 | 13.00 | 8.74 | 2.45 | 24.19 |
| Greenhouse 2..... | 9.0 | 74.6 | .12 | .64 | 13.24 | 26.27 | 4.00 | 43.51 |
| Wheat, variety blue stem, 1926, 48 days old | | | | | | | | |
| Control greenhouse. No heads produced..... | 3.4 | 86.4 | .93 | 3.42 | 13.56 | 2.73 | 2.87 | 19.16 |
| Greenhouse 1. Straw only..... | 3.3 | 76.4 | .47 | 2.15 | 15.46 | 8.07 | 3.26 | 26.79 |
| Greenhouse 2. Straw only..... | 6.3 | 68.2 | .25 | 1.16 | 15.50 | 20.29 | 1.88 | 37.17 |
| 24 hour day. Straw only..... | 1.8 | 64.6 | .29 | 1.15 | 18.11 | 14.13 | 2.93 | 35.17 |
| Greenhouse 1. Heads only..... | .6 | 71.3 | .60 | 2.18 | 25.43 | 28.07 | 6.79 | 60.29 |
| Greenhouse 2. Heads only..... | 2.1 | 64.7 | .51 | 2.11 | 40.53 | 10.83 | 3.12 | 54.48 |
| 24 hour day. Heads only..... | .5 | 61.5 | .26 | 1.26 | 30.01 | 14.14 | 3.80 | 47.95 |

Greenhouse 1 received six hours supplementary light each night from crane.

Greenhouse 2 received same illumination and additional carbon dioxide.

24 hour day plants grown in constant-light room.

TABLE 19. *Chemical Composition of Tobacco and Ragweed, 1925. Aërial Portion*

| Treatment | Wt. per Plant, Grams | Moisture % | Nitrogen, % Dry Weight | | Carbohydrate, % Dry Weight | | | |
|-----------------------------------|----------------------|------------|------------------------|-------|----------------------------|---------|----------|-------|
| | | | Soluble | Total | Acid Hydrolyzable | Sucrose | Dextrose | Total |
| Tobacco, under conditions 38 days | | | | | | | | |
| Control greenhouse..... | 866 | 88.3 | .20 | 1.12 | 21.8 | 2.85 | 7.14 | 31.8 |
| Greenhouse 1*..... | 1230 | 86.6 | .22 | 1.14 | 24.8 | 3.65 | 7.67 | 36.2 |
| Greenhouse 2*..... | 1295 | 84.2 | .15 | .51 | 33.6 | 3.92 | 8.45 | 46.0 |
| Constant-light room*..... | 817 | 85.7 | .21 | 1.28 | 29.0 | 3.62 | 6.47 | 39.1 |
| Ragweed, under conditions 60 days | | | | | | | | |
| Control greenhouse*..... | 260 | 81.1 | .73 | 3.27 | 13.9 | 2.14 | 1.07 | 17.13 |
| Greenhouse 1..... | 333 | 80.2 | .48 | 2.54 | 15.1 | 3.24 | 1.73 | 20.03 |
| Greenhouse 2..... | 598 | 72.5 | .11 | .88 | 17.9 | 12.98 | 1.99 | 32.89 |

* Flowering and fruiting.

Greenhouse 1 received daylight plus six hours additional light from gantry crane each night.

Greenhouse 2 received same illumination as 1 but had about 10 times the normal CO₂ concentration.Constant-light room received continuous artificial illumination plus additional CO₂ as in 2.



TEXT FIG. 23. Clydesdale oats grown at 78° F. Two at left grown in control greenhouse, two at center from gantry crane house with higher concentration of carbon dioxide, two at right same as center except normal carbon dioxide concentration. All plants 45 days old from seed. TEXT FIG. 24. A series of oats similar to FIG. 23 except grown at low temperature, 68° F. The plants at right marked "flue gas" were grown under normal greenhouse conditions except with carbon dioxide concentration about ten times normal. The gas was produced by scrubbing flue gases. All plants 34 days old from seed. TEXT FIG. 25. Blue stem, spring wheat. Two pots at left grown in control greenhouse, two at center with 6 hours supplementary light plus higher carbon dioxide concentration, two at left same as center except normal carbon dioxide concentration. Plants 47 days old from seed. Temperature 78° F. TEXT FIG. 26. Blue stem wheat similar to FIG. 25 except grown at 68° F. Plants 48 days old from seed. Left to right control, House 2 received 6 hours supplementary lighting plus higher concentration of carbon dioxide from steel cylinders, House 1 the same except normal carbon dioxide concentration. Flue gas was grown in a greenhouse with higher concentration of carbon

and without additional light and carbon dioxide is listed. Those plants receiving both additional light and carbon dioxide weigh much more and have greater amounts of carbohydrates as compared to the control plants growing in the greenhouse. The oat plants in the control house were just coming into head when sampled while those in Greenhouse 1 and 2 had been in head several days. The heads were discarded before sampling for analysis. The control wheat plants showed no sign of heading when sampled while those in Greenhouse 1 and 2 had been in head for some time.

Text figure 23 illustrates oat plants (Clydesdale variety) grown at a high temperature in 1925 (78° F.). Figure 24 is a similar series grown at a low temperature in 1926 (68° F.). Those grown at the low temperature have stiff straw and small leaves while those grown at the high temperature have larger and more succulent leaves with a more flexible straw. Those grown with extra light only (gantry crane house 2) in 1926 were shorter and weaker than those grown in the same house in 1925.

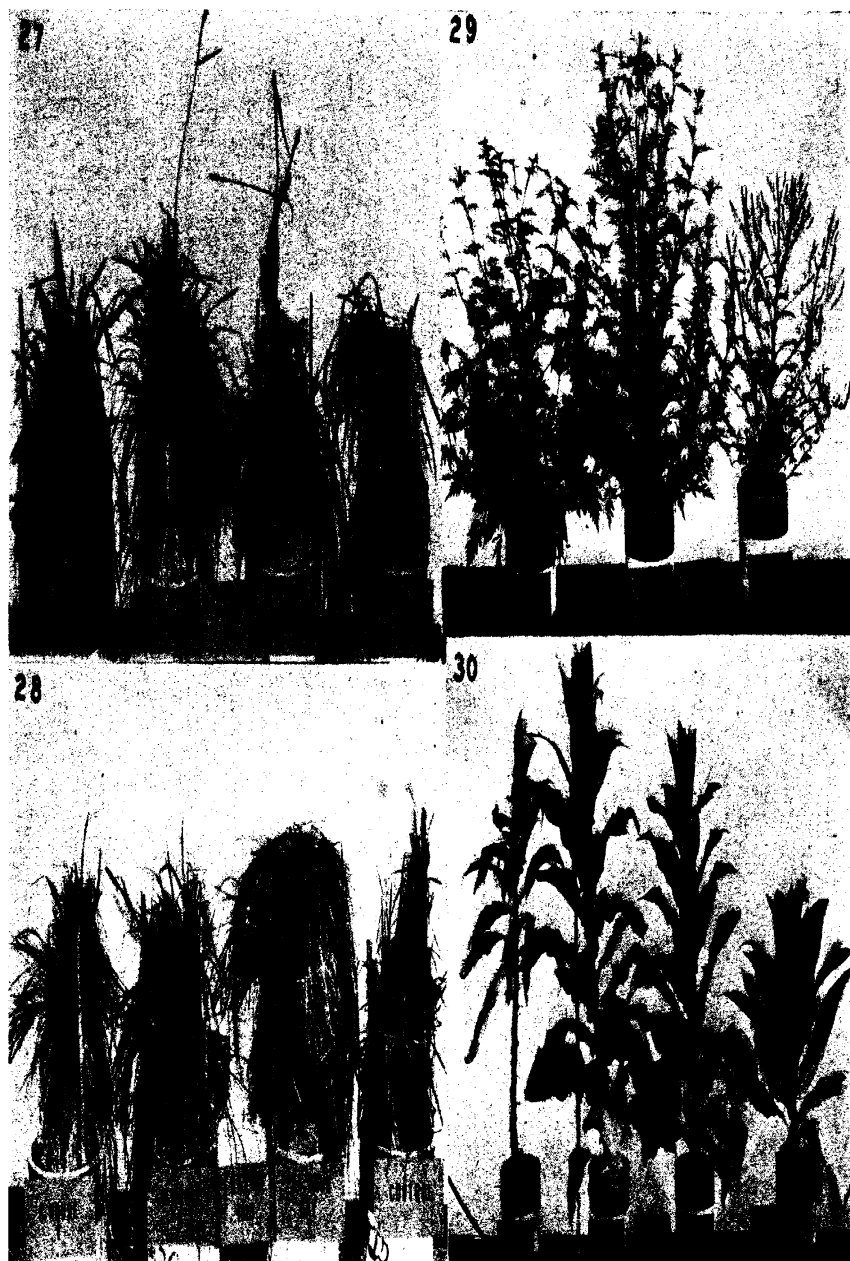
Two varieties of winter wheat were grown, Turkey Red and Hybrid 128, also spring wheat of the Blue Stem variety. Wanser (21) reported that winter wheat required a critical photo-period for jointing and a different photo-period for heading. Schafer, Gaines and Barbee (18) state that Hybrid 128 does not head when planted later than March 11, while Turkey Red planted in April will joint in October while Hybrid 128 will not.

In these experiments Hybrid 128 always produced a few heads on an 18 hour day in the gantry crane house and on a 24 hour day with continuous artificial light. Turkey Red did not head under these conditions but formed dense mats of vegetative growth. Spring wheat was always favored by long days and was grown from seed to head in 31 days in both the gantry crane house 2 and constant-light room in the 1925 experiments. Photographs of these plants are shown in text figures 25, 26, 27, and 28.

Other Plants Grown

Several other species of plants were grown in the gantry crane houses with supplemented light. Of these only tobacco and ragweed (*Ambrosia artemisiifolia*) were analyzed. This data is presented in table 19, while photographs of these plants are reproduced in text figures 29 and 30. The ragweed is a short day plant. It flowered on April 21 after 54 days in the control greenhouse at a height of about 19 inches while it remained vegetative and did not flower in the gantry crane houses on an 18 hour day reaching a height of 40 inches and a weight more than twice as great as the control plants. The weight per plant and total carbohydrates increased with additional light and again with additional gas both with tobacco and ragweed plants. Total nitrogen decreased. This has been found to be true in general with most plants grown in these experiments.

Several ornamental plants such as roses, sweet peas, snapdragon, petunia and nasturtium grew and flowered remarkably well with additional light



TEXT FIG. 27. Winter wheat, Hybrid 128, showing the tendency to head when grown with daylight plus 6 hours of artificial light plus additional carbon dioxide (House 2) or in the constant-light room under continuous illumination. This variety did not head in the control greenhouse or in House 1 with 6 hours of artificial light and the normal carbon dioxide concentration. Plants 66 days old from seed. TEXT FIG. 28. Winter wheat, Turkey Red variety, did not head under any of the conditions. TEXT FIG. 29. Ragweed (*Ambrosia artemisiifolia*) showing the flowering on the short days in the control greenhouse and tendency to remain vegetative in Houses 1 and 2 where the plants received 6 hours additional illumination each night. Plants in House 2 receiving higher concentration of carbon dioxide grew more rapidly than those in House 1 with the normal gas concentration. TEXT FIG. 30. Havana tobacco. Left to right, 24 hour day, Greenhouse 1, Greenhouse 2, and control greenhouse. Tobacco was injured considerably by continuous artificial illumination (24 hour day).

and carbon dioxide. Carnations did not respond to additional light and gas in the 1927 experiments. This may have been due to the comparatively high temperature (78° F.) since carnations are known to grow best at a low temperature. Hoosier Beauty, Premier, and roses of the rambler type all flowered profusely with additional light and carbon dioxide. Hoosier Beauty and Premier sent up new canes from the root-stock which produced clusters of two or three flowers in less time than was required for flowers to develop from existing canes in the control plants at this season of the year. One of the common effects of these conditions was the production of two or three large blooms on a single cane at one time. This effect is shown in text figure 31. In the control plants normally only one bud opens at a time on each cane. The rose flowers grown with additional light at 68° F. in 1927 shown in figure 31 were of better keeping quality than those grown at 78° in the same year but it required longer for them to develop. They still retained at the low temperature the characteristic of opening two or three flowers in a cluster at one time which was found to be true at the high temperature when grown with both additional light and carbon dioxide.

Nasturtium flowered profusely in Greenhouse 2 with additional light and carbon dioxide. Additional light alone was little better than the control conditions in forcing flowers. The great amount of flowering in House 2 is shown in text figure 32. The plants are all 69 days old from seed. The first flowers appeared in House 2 when the plants were 38 days old and in both House 1 and the control at 52 days of age. In the final yield of flowers during the experiments House 2 was first, House 1 second, and the control lowest. Nasturtium plants grew more rapidly at the higher temperature in 1925 and flowered earlier. In the low temperature series of 1926 House 2 plants flowered first at the age of 54 days and House 1 and control plants one week later.

Eggplants with additional light and gas (House 2) grew very rapidly and set several large fruits. Plants grown in House 1 were second in amount of fruit set. The controls did not fruit during the experiment. Text figure 33 shows the plants grown under the different conditions. The plants were in the conditions 67 days when the photograph was taken. All were small plants about three inches high when the experiments were started on February 28.

Tomato has been mentioned as an outstanding example of a plant which does not withstand continuous artificial illumination. Geranium and coleus also fall naturally into this group, the only difference being in the degree of injury. Photographs of these two species are shown in text figures 33 and 34. The injury of continuous illumination is apparent in both cases. These two species always managed to survive during the period of the experiments but they were always reduced to a stem with only a few small leaves remaining alive. Geranium, in contrast to tomato,



TEXT FIG. 31. Premier and Hoosier Beauty roses grown in gantry crane house with additional light and carbon dioxide showing the effect in forcing clusters of three sturdy roses at one time. New canes with flowers were produced from the root-stock in about the same time required to produce a single flower bud from existing canes in the control plants. TEXT FIG. 32. Nasturtium plants 70 days old from seed. The one in the center receiving both additional light and gas is flowering profusely. The plants from Greenhouse 1 receiving additional light only are little better than the controls (at right) on the normal length of day. TEXT FIG. 33. Geranium showing the flowering with supplementary light in Greenhouses 1 and 2 and the injury of continuous illumination (24 hour day). The control plant is at the right. TEXT FIG. 34. Variegated coleus, showing the increased growth with both additional light and gas (Greenhouse 2) as compared with additional light only (Greenhouse 1) at left and control greenhouse at right. The 24 hour day plant shows considerable light injury. TEXT FIG. 35. Eggplant showing the additional growth and fruiting with additional light only (Greenhouse 1) and with both light and gas (Greenhouse 2 center). The control at right did not fruit during the experiments.

grew well with continuous illumination in the gantry crane house in the 1927 experiments with 12 hours of daylight in combination with 12 of artificial light. This may be due to the difference in quality of the light source, sunlight always being more favorable for plant growth than that of the filament lamp. It may also be due to short periods of recovery at low intensities of daylight in the late afternoon or on cloudy days in February when the artificial light source was off, while those under continuous artificial illumination had practically no rest period or appreciable decrease in intensity during the entire period of the experiment.

PRACTICAL APPLICATION

These experiments were undertaken to determine some of the effects of environmental factors on plant growth rather than the possible application of such information as might be obtained to the commercial growing of plants in greenhouses. However, it is believed that some items of cost should be included in this report for the information of those interested in reproducing conditions similar to those already outlined for growing plants. 124 jars of the two gallon size were filled with soil and placed in the constant-light room for each experiment. One or more plants, depending upon size, were grown in each jar. Assuming that each jar contained only one plant 124 plants can be grown on a 24 hour day during each experiment with artificial light only. The cost for refrigeration, steam, and operation of four motors together with the attendants amounted to about \$26.00 per 24 hour day. The cost for lighting current at four cents per kilowatt hour was \$36.00 per day or about 29 cents per jar of plants. The total cost of operation therefore was \$62.00 per day. The 1925 experiments were continued for 75 days making a total cost of \$4650.00 or about \$37.00 per jar of plants. The total cost of lighting current alone was \$21.00 per jar. The additional item of carbon dioxid from tanks cost about \$8.00 per day and is not included in these calculations. The calculations also do not include the original cost of machinery and equipment, or depreciation. Many plants were grown throughout their life history in less time than 75 days. It has already been pointed out that many plants produce very little additional growth on a 24 hour day as compared to an 18 hour day, and also that some plants such as the tomato grow better at a lower light intensity. The cost calculations above could be reduced considerably therefore if a practical application were the aim.

It is believed that some application will be found, however, in supplementing daylight with artificial light for a period of three to six hours each night rather than in the use of artificial light entirely. In the case of the gantry crane the cost per day for six hours of supplementary lighting was \$11.52. 130 jars of plants were grown at a cost of about nine cents per day per jar or at a total cost of \$6.75 for the entire 75 days. A number of plants could be brought into full production with the intensities used

in the gantry crane houses during these experiments in 40 days, and since electric power could be used during the early morning hours when there is a small load on the lines it could be obtained for at least half of the price per kilowatt indicated above. The estimated cost would then reduce to about \$1.80 per jar for the entire growth period. This does not include the cost of lamps, equipment, or carbon dioxid.

DISCUSSION AND SUMMARY

This report is concerned with the growth of plants in artificial climates. Some of the plants were grown with artificial light only as a source of energy for photosynthesis. Other plants were grown with daylight supplemented with artificial light for six to 12 hours each night. An attempt was made to grow plants throughout their life history with photosynthesis at or near its maximum rate by supplying a high light intensity and long day along with increased carbon dioxid concentration and a relatively high temperature. The effect of length of day on certain species was also studied in various combinations of temperature and carbon dioxid supply. Chemical analyses of many plants grown under the different conditions are given, together with a discussion of the effect of various factors on the percentage carbohydrate and nitrogen in various tissues.

There is a difference in the percentage carbohydrate in tomato leaves, depending on the time of day at which they are sampled. When allowed to remain in darkness for 17 hours after exposure the total carbohydrate and especially the sucrose and dextrose fractions decrease considerably. After 40 hours in darkness these fractions decrease to approximately one-third of the original value. Depending upon when the plants are sampled, in relation to their light exposure period, various values for the carbohydrate-nitrogen ratio may be obtained. This variation is due to changes in carbohydrate since total nitrogen remains practically the same.

No relation was found between carbohydrate and nitrogen content and flowering in either long day plants such as radish and lettuce, or in salvia, a short day plant, or buckwheat, an everblooming type. It was found that the percentages of carbohydrate and nitrogen in general could be changed by varying light intensity, length of day, or in some plants by changing the nutrient supply when the plants were grown in sand instead of soil. The range of variation of these fractions depends upon the plant species. For salvia the range of total carbohydrate on a dry weight basis is narrow, since even the five hour day plants are able to maintain a high level of total carbohydrates. Total nitrogen also was restricted to a comparatively narrow range from 5 to 19 or 24 hour days. The application to the soil of large quantities of nitrate made practically no difference in the total nitrogen content of the aerial portion of salvia plants although it resulted in considerable foliar injury. This plant is apparently able to hold both the carbohydrate and nitrogen fractions within a narrow range when grown

under conditions which greatly affect the range in lettuce, radish and buckwheat. *Salvia* plants were kept from flowering by illumination for six hours each night from January to December with very little change in either the carbohydrate or nitrogen fractions.

The tomato was found to be the most light sensitive of any plant grown in these experiments. It will not withstand a 24 hour day at an intensity which causes little or no injury to other plants. The plants set fruit on all day lengths from seven to 19 hours but did not fruit on either five or 24 hour days. Fruit production and weight per plant were maximum in rapidly growing high carbohydrate, low nitrogen plants grown with daylight plus six hours of artificial light each night and with increased carbon dioxid supply. At higher intensities day lengths of 17 and 19 hours are injurious. When the plants are receiving 12 hours of artificial light at night more than six hours of sunlight is injurious. In general carbohydrates and weight per plant increase with day length up to the point where foliar injury begins to be effective in holding the plants back. At a low light intensity this increase holds up to a 17 or 19 hour day, while at higher light intensities the peak is reached on a 12 hour day. On long days total nitrogen was decreased to less than one percent of the dry weight. On a percentage of dry weight basis the ratio of total carbohydrate to total nitrogen closely parallels total carbohydrate. It is seen, therefore, that there is little relation between this ratio and the setting of fruit.

It is thought that the long day injury to tomato plants is produced by a breaking down in the process of photosynthesis rather than by too great an accumulation of the products of the process, since the injury can be produced with a low light intensity which results in no accumulation of carbohydrates in the leaves as compared to greenhouse plants.

Cabbage plants were found to increase in weight of tissue produced and in total carbohydrate with length of day up to 17 or 19 hours followed by a decrease on continuous illumination. The percentage total carbohydrate was usually doubled on a 19 hour day as compared to a five hour day. On long days this plant produced over 50 percent of the dry weight in easily available carbohydrates. Total nitrogen decreased with day length to 19 hours and increased slightly on a 24 hour day.

Red clover grew and flowered especially well with daylight supplemented by six hours of artificial light from the gantry crane in the greenhouses where carbon dioxid concentration was increased. Plants were grown from seed to flower in 38 days during the month of March and the first week in April while the control plants did not flower during the experiments. Carbohydrates increased and nitrogen decreased with increasing day length and increased carbon dioxid supply.

Four varieties of soy beans, Mandarin, Peking, Tokio and Biloxi, which Garner and Allard had observed to flower in June, July, August, and September respectively, were found to flower in the control greenhouse.

Of these only Mandarin and Biloxi flowered with additional light and carbon dioxid. Carbohydrates and weight per plant increased and nitrogen decreased with additional light and carbon dioxid.

Cucumber plants were found to absorb nitrate readily in contrast with salvia which absorbs little of this salt. This was especially true on the long days where a shortage of nitrogen had been produced due to an accumulation of carbohydrates. Total carbohydrates and weight per plant increased with additional light and higher concentrations of carbon dioxid.

Tuber production in potatoes, variety Irish Cobbler, was found to be favored by low temperature in combination with high light intensity and long days. High temperature (78° F.) produced weak stems with little or no tuberization, although weight of the aerial portion produced at high temperature was usually greater than at low temperature (68° F.). This variety formed many large tubers when grown with continuous illumination. The failure of previous workers to get tuberization on a long day is believed to be due to the high temperature conditions which were associated with long days in their experiments. Since high light intensity, long days, and cool air temperature greatly favor tuber production it is thought that these factors account for the high yields of potato tubers in high latitudes and higher altitudes.

Small grains such as barley and spring wheat, in contrast to potatoes, will grow well and yield well at a high temperature (78° F.) if given additional light and carbon dioxid. The production of these grains is not favored by low temperature when day length is long and carbon dioxid supply is abundant. The weight per plant of barley increases with day length up to a 19 hour day. Total carbohydrates also increase and nitrogen decreases. The feeding of nitrate was found to make little or no difference in the total percentage of nitrogen in the barley plant, the percentage remaining high only when carbohydrate synthesis was restricted by short days.

Winter wheat of the Turkey Red variety, did not head in these experiments, while Hybrid 128, a second variety of winter wheat, produced several heads on 18 and 24 hour days. Spring wheat, variety Blue Stem, headed especially well with additional light and carbon dioxid. This variety was grown from seed to head in 31 days.

Several ornamental plants such as roses, sweet peas, snapdragons, petunia, and nasturtium grew and flowered remarkably well with additional light and carbon dioxid. Both geranium and coleus were greatly injured, however, by continuous artificial illumination, the injury being similar to tomato but not quite as severe. In contrast to tomato these plants could be grown with little or no injury with continuous illumination if sunlight was used as a light source during the day and was supplemented with 12 hours of artificial light each night. Sunlight, in general, is a better light source for plant growth than the incandescent filament lamp.

A practical application of artificial light to the growing of plants will no doubt be found in supplementing daylight during the winter months with three to six hours of artificial light each night. The cost of growing plants with artificial light alone is prohibitive except for experimental or demonstrational purposes. In addition sunlight is a better source of energy for growing plants than artificial sources now available.

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ENZYM ACTIVITIES OF JUICES FROM POTATOES TREATED WITH CHEMICALS THAT BREAK THE REST PERIOD ^{1, 2}

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INTRODUCTION

When freshly-harvested potatoes are treated with chemicals such as ethylene chlorhydrin, sodium thiocyanate, and thiourea the rest period is broken, and sprouts begin to make their appearance uniformly throughout the lot, after about five to eight days. The untreated potatoes planted at the same time, however, usually do not show sprouts until several weeks later, and even then the sprouting is not uniform (4).

It seemed desirable to study the enzym changes that take place in the potatoes during these few days in which the processes correlated with the breaking of dormancy are in progress, and to compare them with untreated tubers under the same conditions. This paper presents the results of a series of measurements of the enzym activities of juices obtained from potatoes which had been treated with chemicals but which had not yet sprouted.

The three chemicals used for treating the potatoes were ethylene chlorhydrin ($\text{ClCH}_2\text{CH}_2\text{OH}$), sodium thiocyanate (NaSCN), and thiourea (NH_2CSNH_2). These, although quite unlike in chemical character, produce similar results in the breaking of dormancy and therefore it seemed of special interest to compare them with regard to their effects upon enzym activity.

The object of the experiment was to obtain partial or complete answers to such questions as the following: What enzymes show the greatest changes? How soon after treatment do the changes start? Do the different chemicals produce the same or different effects upon the enzymes? Is there any relation between the concentration of chemical used in treating the potato and the enzym activity of the press-juice? Is there any correlation between the sprouting response and the enzym changes? Are the chemicals acting directly upon the enzymes or do the chemicals act first upon the living matter and only indirectly upon the enzymes? Do the changes occur in the absence of the eyes or must these be present to permit a response?

The enzymes mainly studied in this series of experiments were catalase,

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peroxidase, and oxidoreductase; a few tests with amylase and invertase were made; and in the course of the tests it was found that the pH changes and certain non-enzymic reducing properties of the juice had to be taken into account.

A general statement of the results is as follows: Increases in various enzymes were induced by the treatments and in some cases these were detectable within 24 hours. The amount of the increase was related to the concentration of the chemical used, in such a manner that a series of chemical treatments of graded strength gave a corresponding gradation of enzyme activities. The three different chemicals were not equal in their effect upon enzymes, ethylene chlorhydrin being much more effective than sodium thiocyanate and thiourea. The correlation between the sprouting produced by the treatment and the amount of increase in enzyme activity was not close, the thiourea treatments, particularly, producing better sprouting than would be expected from the effect upon enzymes. Presence of eyes in seed-pieces treated with chemicals was not necessary for increases in enzyme activity. Increases could not be obtained by adding the chemicals to the press-juice; they occurred only when the potatoes were treated with chemicals and press-juices obtained at a subsequent period.

MATERIALS AND METHODS

Varieties and Source of Seed

Tubers of both Irish Cobbler and Bliss Triumph varieties were used. Freshly harvested potatoes of Irish Cobbler were obtained early in June from Mr. C. E. McLeod, Jr., of Seabrook, S. C., late in June from Dr. R. A. McGinty of Clemson College, S. C., and in mid-July from Mr. J. M. Snyder of College Park, Maryland. To these persons and to Dr. C. O. Appleman of the University of Maryland the writers wish to express thanks for their valuable coöperation in obtaining a dependable supply of experimental potatoes. For the tests later in the season tubers of Bliss Triumph and Irish Cobbler harvested in August from the Institute gardens were used. The Bliss Triumph seed potatoes from which this crop was grown were kindly furnished by the Nebraska Certified Potato Growers Association through the courtesy of Mr. William Morrow and Prof. H. O. Werner of the University of Nebraska, to both of whom we wish to make grateful acknowledgment. The response of tubers of these different varieties from different localities was not noticeably dissimilar so far as the enzyme studies were concerned. Bliss Triumph has a shorter dormant period than Irish Cobbler and responds somewhat more readily to chemical treatment but the changes observed were always in the same direction and approximately equal in amount in the various lots. A much greater divergence was found between the results with whole tubers and cut tubers of the same variety than between varieties.

Chemical Treatments

The methods used in treating the potatoes were the same as those described in previous publications (4, 5). The ethylene chlorhydrin "dip method" consisted in dipping the cut tubers (one-eye pieces weighing about one ounce) into a dilute solution of ethylene chlorhydrin (the exact amounts of 40 percent ethylene chlorhydrin per liter being shown in the tables), and storing the dipped pieces in covered glass jars for a definite period (usually 24 hours but sometimes only 16 or even eight hours). At the end of the storage period the treated pieces were planted in soil in flats until a subsequent time at which it was desired to remove a sample for the tests. The check lot consisted of pieces from the same lot of tubers, stored for the same length of time in the same type of containers, and handled in exactly the same manner except that they were dipped into water instead of chemical solutions. The sodium thiocyanate and thiourea treatments consisted in soaking the potatoes (cut into pieces ready for planting) for one hour in the solutions, the strength of which is described in the tables, and then planting the treated pieces in soil at once without rinsing. The chlorhydrin treatment for whole tubers consisted in placing whole tubers in glass or earthenware vessels for which covers were provided, exposing them for one day to vapors of ethylene chlorhydrin which was allowed to evaporate from pieces of cheesecloth placed loosely at the top of the vessel. The treated tubers were then stored intact (without cutting into pieces) in paper bags until samples for the tests were wanted.

Obtaining Samples

In taking samples for analysis 25 to 50 of the cut pieces were removed from the soil, were wiped with a moist rag; then the eye of each piece was removed by first cutting away the flesh to the level of the eye and picking out the eye with a knife point, taking about one-tenth gram of tissue per eye; from the rest of the seed-piece a thin layer of the outer surface was removed. The eye-tissue was ground up in a mortar using successive quantities of added water, decanting after each grinding, until the final volume including the residue was 15 cc. for each gram of tissue taken; samples of the not-at-eye portion (called in the tables "tissue exclusive of eye-tissue") were obtained by cutting off a slice from each seed-piece until 50 to 75 grams of tissue were collected; the tissue was placed in a small cheesecloth bag, pounded in a mortar and pressed in a hand-press; the bag was again pounded and pressed; three pressings were made in all, the volume of juice obtainable being about 0.5 cc. per gram of tissue taken. The juice was not allowed to stand after pressing but samples for the various tests were taken within a few minutes.

It was found necessary to change this procedure for part of the work as follows: (1) Juice used for the study of both enzymic and non-enzymic reducing substances could not be subjected to so much aëration as this

method entailed, and for such tests the juice was obtained by passing the tissue through a food grinder, placing it in a cheesecloth bag, and pressing by hand. (2) It was found, as shown later in the tables, that the treatments, especially those with ethylene chlorhydrin, produced a juice with a pH different from that of the check lot; therefore special pressings were made in which the tissue was rolled in calcium carbonate previous to pressing.

RESULTS

Catalase

For the catalase determinations, usually two cc. of juice were diluted to 50 cc. with water and ten cc. of this were taken for the determination. In certain cases a different dilution ratio had to be chosen in order to give a suitable burette reading but in such cases the check juice was proportionately diluted. The apparatus and procedure described by Davis (3) were used except that the Dioxygen was neutralized by CaCO_3 instead of NaOH .

The effect upon catalase obtained by treating potatoes with different concentrations of chemicals is shown in tables 1, 3, and 6, which refer respectively to ethylene chlorhydrin, sodium thiocyanate, and thiourea.

Columns 5 and 12 show that the effect of the ethylene chlorhydrin treatment was to increase greatly the catalase activity of the press-juice. Thus, favorable concentrations of chlorhydrin approximately doubled or even trebled the catalase in the cut-tuber dip-method treatments, and in the case of the whole-tuber treatments the differences between treated and check are even greater. It will be observed that with few exceptions when a graded series of chemical concentrations was used in treating the potatoes, a correspondingly graded series of catalase determinations was obtained. This is especially true of the juices obtained from the eye-tissue. It will be noted that the check readings for the whole-tuber series were lower than the checks from the cut-tuber series. Merely cutting the tuber and planting the pieces increased the catalase of the press-juice. Although, as shown in table 1, column 10, the pH of the press-juice (and even that of the juice after the dilution of 2 : 50 for the catalase determination, see column 11), was higher (less acid) for the treated than for the check, and therefore was more favorable for the catalase of the treated than of the check, the differences in catalase activity can not be explained by this difference in pH; for, when samples of both treated and check juices were brought to various pH values by the addition of acid or alkali the catalase of the treated was higher than the check at any pH value within the range of acidity encountered. Thus, in table 2 are shown the results of adjusting the juices of treated and check samples to various pH values and then determining the catalase activity. The treated juice was then found to be more active at the greatest acidity than the check juice was at the lowest acidity. Subsequent experiments showed that, even when ground with calcium carbonate in order to neutralize the acidity, the lots treated with

TABLE 1. *Effect of Different Concentrations of Ethylene Chlorhydrin Upon Dormant Potato Tubers*

| Treatment | Lot No. | Conc. of Chemical per Liter | Per-cent Germ. | Juice from Tissue Exclusive of Eye-Tissue | | | | | | | Juice from Eye-Tissue Only | | | Days After Treatment Sample Taken |
|---|---------|-----------------------------|----------------|---|--------------------------------|-------------|---|---------------------------|--------------|----------------|---------------------------------------|--------------------------------|-------------|-----------------------------------|
| | | | | Catalase, cc. O ₂ in 1 Min. | Peroxidase Ratio Treated Check | | Methylene Blue Reduction Minutes Required | Indo-phenol cc. Ab-sorbed | pH of Juice* | | Catalase cc. O ₂ in 1 Min. | Peroxidase Ratio Treated Check | | |
| | | | | | Purpuro-gallin Method | Nadi-method | | | As Pressed | After Dilution | | Purpuro-gallin Method | Nadi-method | |
| Chlorhydrin, cut tubers, dip method, Cobbler | 207 | 45 cc. | 90 | 14.3 | 1.77 | 1.38 | 1.0 | 11.5 | 6.51 | 6.78 | 20.6 | 2.13 | 1.20 | 4 |
| | 208 | 15 cc. | 70 | 19.1 | 1.80 | 1.04 | 6.0 | 5.0 | 6.34 | 6.78 | 17.0 | 2.27 | 1.70 | |
| | 209 | 5 cc. | 27 | 16.7 | 1.57 | 1.11 | 1.0 | 7.0 | 6.34 | 6.68 | 12.8 | 1.49 | 1.11 | |
| | 210 | Check | 0 | 8.0 | 1.00 | 1.00 | Neg. | 2.5 | 6.03 | 6.56 | 5.9 | 1.00 | 1.00 | |
| Chlorhydrin, cut tubers, dip method, Cobbler | 252 | 45 cc. | 96 | 21.7 | | | | | | | 19.5 | | | 4 |
| | 253 | 15 cc. | 64 | 21.2 | | | | | See Note | | 15.0 | | | |
| | 254 | 5 cc. | 40 | 16.8 | | | | | | | 10.6 | | | |
| | 255 | Check | 8 | 13.3 | | | | | | | 10.0 | | | |
| Chlorhydrin, whole tubers, vapor meth-od, Cobbler | 137 | 1.00 cc. | 58 | 20.5 | 13.24 | 2.10 | 0.5 | 36.0 | 7.24 | 6.90 | 13.5 | | | 5 |
| | 138 | 0.20 cc. | 60 | 9.3 | 4.40 | 1.69 | 1.5 | 14.0 | 6.56 | 6.90 | 14.3 | | | |
| | 139 | 0.04 cc. | 13 | 3.2 | 2.11 | 1.24 | Neg. | 4.0 | 6.17 | 6.73 | 9.2 | | | |
| | 140 | 0.008 cc. | 0 | 2.8 | 1.20 | 0.94 | Neg. | 2.0 | 6.05 | 6.64 | 5.8 | | | |
| | 141 | Check | 0 | 1.6 | 1.00 | 1.00 | Neg. | 2.5 | 6.00 | 6.56 | 3.9 | | | |
| Chlorhydrin, whole tubers, vapor meth-od, Bliss | 240 | 0.50 cc. | 66 | 18.9 | 2.82 | Lost | | | | | 21.7 | 3.37 | | 7 |
| | 241 | 0.16 cc. | 20 | 9.1 | 2.50 | 2.11 | | | | | 10.0 | 2.36 | | |
| | 242 | 0.06 cc. | 0 | 4.3 | 1.46 | 1.55 | | | | | 7.5 | 2.14 | | |
| | 243 | Check | 12 | 2.9 | 1.00 | 1.00 | | | | | 3.9 | 1.00 | | |
| Chlorhydrin, whole tubers, vapor meth-od, Bliss | 193 | 1.00 cc. | 59 | | 2.15 | | 0.5 | | | | | | | 7 |
| | 194 | 0.25 cc. | 18 | | 1.38 | | 8.0 | | | | | | | |
| | 195 | 0.06 cc. | 27 | | 1.09 | | 30.0 | | | | | | | |
| | 196 | Check | 0 | | 1.00 | | Neg. | | | | | | | |

Note: Tissue for lots 252-255 ground with calcium carbonate.

* pH measurements made with quinhydrone electrode.

ethylene chlorhydrin had a greater catalase activity than the corresponding checks.

TABLE 2. *Effect of pH Upon the Catalase Activity of Potato Juice*

| | | Catalase, cc. O ₂ in 1 min. | |
|----------------------|-----------------|--|------------------------------|
| | | Juice from Treated Potatoes | Juice from Check Potatoes |
| Juice adjusted to pH | 5.96..... | 12.8 | 1.4 |
| " | " " " 6.10..... | 16.4 | 2.9 |
| " | " " " 6.50..... | 18.3 | 5.4 |
| " | " " " 6.95..... | 19.7 | 7.7 |

The pH of the juice during the process of pressing and making the catalase measurement is not capable of explaining the increased activity of the juice from the treated lots; but the higher pH value of the sap which may have prevailed in the potato between the time of treatment and time of sampling may be of great importance in protecting the catalase formed or in inducing a greater production of catalase in the treated lots. These experiments throw no light on this point.

The increases in catalase in potatoes treated with ethylene chlorhydrin were not due to the direct effect of the ethylene chlorhydrin upon the catalase already present. This was shown by experiments in which various amounts of ethylene chlorhydrin were added to potato juice and the catalase activity subsequently measured. In no case were any increases observed; decreases were found if the amount of chemical added was large enough. Thus eight cc. of 40 percent ethylene chlorhydrin per 100 cc. of potato juice caused a decrease in the catalase, but when the amount added was less than about two cc. per 100 cc. of juice no effect upon the catalase was noted.

The effect of sodium thiocyanate treatment of potatoes upon the catalase of press-juice is shown in table 3, columns 5 and 12. In the determinations with juice from tissue exclusive of eye-tissue (column 5) it is seen that small and probably insignificant differences were found between the check lots and the lots treated with various concentrations of chemical. Only when we examine the data on juices from eye-tissue, column 12, do we find any suggestion that the catalase activity has been increased, and, even here, the differences are small, and hardly beyond the experimental error.

Preliminary experiments had shown that the addition of small amounts of sodium thiocyanate to potato juice greatly depressed the catalase activity, even ten milligrams of NaSCN per 100 cc. of potato juice causing a marked reduction. It was thought possible that, in the process of soaking the potatoes in sodium thiocyanate solutions, enough chemical could be present in the press-juice to have a retarding influence upon catalase. Tests showed that thiocyanate was present in appreciable amounts in the juice from the treated potatoes. Consequently, the juices were dialyzed in collodion bags in running water, under which conditions the catalase is retained within the bag and the thiocyanate together with other easily diffusible substances passes through. This permitted a separation of the

TABLE 3. *Effect of Different Concentrations of Sodium Thiocyanate Upon Dormant Potato Tubers*

| Treatment | Lot No. | Conc. of Chemical Percent | Per-cent Germ. | Juice from Tissue Exclusive of Eye-Tissue | | | | | | Juice from Eye-Tissue Only | | | Days After Treatment Sample Taken | |
|------------------------------------|---------|---------------------------|----------------|---|--------------------------------|-------------|---|---------------------------|--------------|----------------------------|--|--------------------------------|-----------------------------------|-------------|
| | | | | Catalase, cc. O ₂ in 1 Min. | Peroxidase Ratio Treated Check | | Methylene Blue Reduction Minutes Required | Indo-phenol cc. Ab-sorbed | pH of Juice* | | Catalase, cc. O ₂ in 1 Min. | Peroxidase Ratio Treated Check | | |
| | | | | | Purpuro-gallin Method | Nadi-method | | | As Pressed | After Dilution | | Purpuro-gallin Method | | Nadi-method |
| Cut tubers, soaked 1 hour, Cobbler | 236 | 1.00 | 100 | 9.0 | 2.15 | 1.59 | 30.0 | 11.0 | | | 9.3 | 2.38 | 1.59 | 5 |
| | 237 | 0.50 | 80 | 8.2 | 1.77 | 1.20 | Pos. | 8.0 | See Note | | 6.9 | 1.79 | 1.20 | |
| | 238 | 0.25 | 65 | 8.7 | 1.21 | 1.20 | Sl. | 2.5 | | | 7.3 | 2.02 | 1.20 | |
| | 239 | Water | 0 | 8.5 | 1.00 | 1.00 | Neg. | 2.5 | | | 6.5 | 1.00 | 1.00 | |
| Cut tubers, soaked 1 hour, Bliss | 182 | 1.00 | 100 | 7.3 | 2.31 | 1.20 | Pos. | 10.0 | 6.10 | 6.64 | 9.0 | 1.50 | 1.19 | 4 |
| | 184 | 0.50 | 84 | 7.2 | 1.40 | 1.32 | Sl. | 8.0 | 5.97 | 6.68 | 11.2 | 0.89 | 0.92 | |
| | 186 | 0.25 | 68 | 6.0 | 1.40 | 0.92 | Pos. | 2.5 | 5.88 | 6.75 | 7.1 | 1.19 | 0.92 | |
| | 183 | Water | 12 | 6.4 | 1.00 | | Neg. | | 5.93 | 6.64 | 8.5 | 1.00 | | |
| | 185 | Water | 16 | 8.2 | 1.36 | 1.00 | Neg. | 2.5 | 5.88 | 6.68 | 7.5 | 0.99 | 1.00 | |
| Cut tubers, soaked 1 hour, Bliss | 167 | 1.00 | 100 | 11.2 | 1.50 | 1.57 | 15.0 | 6.5 | 5.97 | 6.51 | 6.3 | 1.34 | 1.32 | 5 |
| | 168 | 0.50 | 84 | 10.5 | 1.36 | 1.64 | Pos. | 4.0 | 5.88 | 6.44 | 5.8 | 1.03 | 1.08 | |
| | 169 | 0.25 | 60 | 6.8 | 1.04 | 1.22 | Sl. | 2.5 | 5.83 | 6.44 | 3.2 | 0.87 | 1.02 | |
| | 170 | 0.13 | 40 | 7.6 | 0.90 | 1.10 | Neg. | 1.0 | 5.68 | 6.22 | 2.6 | 0.77 | 0.81 | |
| | 171 | Water | 8 | 7.1 | 1.00 | 1.00 | Neg. | 1.5 | 5.97 | 6.31 | 4.9 | 1.00 | 1.00 | |
| Cut tubers, soaked 1 hour, Cobbler | 157 | 2.00 | | 3.5 | 1.48 | 1.44 | Pos. | | 6.00 | 6.47 | 3.9 | | 1.16 | 4 |
| | 158 | 0.67 | | 5.5 | 1.26 | 1.19 | Sl. | | 5.93 | 6.31 | 5.5 | | 1.12 | |
| | 159 | 0.22 | | 6.3 | 1.24 | 1.15 | Neg. | | 6.00 | 6.31 | 5.2 | | 1.04 | |
| | 160 | Water | | 5.1 | 1.00 | 1.00 | Neg. | | 5.97 | 6.31 | 3.2 | | 1.00 | |

Note: Tissue for lots 236-239 ground with calcium carbonate for the catalase determination.

* pH measurements made with quinhydrone electrode.

catalase and the thiocyanate which, if present, could retard it. The results are shown in table 4 from which it can be seen that, although before dialysis there was no graded series of catalase readings corresponding to the series of concentrations of sodium thiocyanate, after dialysis the catalase activities

TABLE 4. *Effect of Dialysis of Potato Juice From Tubers Treated With Chemicals*

| Treatment Applied | Lot No. | Conc. of Chemical Used | Catalase, cc. O ₂ in 1 Min. | |
|--|---------|------------------------|--|----------------|
| | | | Before Dialysis | After Dialysis |
| NaSCN, cut tubers, soaked one hour, Cobbler | 236 | 1.00% | 9.0 | 15.9 |
| | 237 | 0.50% | 8.2 | 12.0 |
| | 238 | 0.20% | 8.7 | 8.4 |
| | 239 | Water | 8.5 | 5.8 |
| Thiourea, cut tubers, soaked one hour, Bliss | 224 | 1.00% | 9.1 | 8.8 |
| | 225 | 0.50% | 9.7 | 8.8 |
| | 226 | 0.25% | 9.8 | 8.2 |
| | 227 | Water | 9.5 | 8.4 |
| Chlorhydrin, whole tubers, vapor method, Bliss | 240 | 0.50 cc. | 18.9 | 19.8 |
| | 241 | 0.16 cc. | 9.1 | 10.0 |
| | 242 | 0.06 cc. | 4.3 | 3.8 |
| | 243 | Check | 2.9 | 2.9 |

then arranged themselves in a series in an order agreeing with the strength of the chemicals.

Further tests were made to determine the effect of length of dialysis upon the catalase activity of juices from the sodium thiocyanate treatments. These results are given in table 5 which shows that a period of dialysis as short as one-half hour greatly increased the catalase of the juice from thiocyanate-treated tubers, and that the maximum effect was reached in about two hours. Dialysis for 16 hours reduced the catalase of both treated and check, but the treated remained higher than the check.

TABLE 5. *Time Relations in Dialysis of Juice From Potatoes Treated With Sodium Thiocyanate*

| Time After Beginning of Dialysis | Catalase, cc. O ₂ in 1 Min. | | | |
|----------------------------------|--|-------|---------|-------|
| | Lot I | | Lot II | |
| | Treated | Check | Treated | Check |
| Start..... | 6.0 | 13.6 | 7.0 | 7.4 |
| ½ hour..... | 15.7 | 14.3 | | |
| 2 hours..... | 19.9 | 14.9 | 18.6 | 11.8 |
| 4 hours..... | 20.0 | 11.6 | 14.7 | 10.0 |
| 16 hours..... | 11.0 | 4.7 | 9.7 | 5.2 |

These results indicate that the effect of the sodium thiocyanate solution upon the catalase of potato was two-fold: (1) It induced the formation of

TABLE 6. *Effect of Different Concentrations of Thiourea Upon Dormant Potatoes*

| Treatment | Lot No. | Conc. of Chemical Percent | Per-cent Germ. | Juice from Tissue Exclusive of Eye-Tissue | | | | | | | Juice from Eye-Tissue Only | | | Days After Treatment Sample Taken |
|------------------------------------|---------|---------------------------|----------------|---|--------------------------------|-------------|---|---------------------------|--------------|----------------|---------------------------------------|--------------------------------|-------------|-----------------------------------|
| | | | | Catalase, cc. O ₂ in 1 Min. | Peroxidase Ratio Treated Check | | Methylene Blue Reduction Minutes Required | Indo-phenol cc. Ab-sorbed | pH of Juice* | | Catalase cc. O ₂ in 1 Min. | Peroxidase Ratio Treated Check | | |
| | | | | | Purpuro-gallin Method | Nadi-method | | | As Pressed | After Dilution | | Purpuro-gallin Method | Nadi-method | |
| Cut tubers, soaked 1 hour, Cobbler | 228 | 1.00 | 96 | 12.7 | 1.50 | 1.41 | Neg. | 3.0 | | | 22.6 | 2.47 | | 5 |
| | 229 | 0.50 | 72 | 12.4 | 1.44 | 1.38 | Neg. | 5.5 | See Note | | 26.0 | 1.47 | | |
| | 230 | 0.25 | 8 | 13.2 | 1.37 | 1.20 | Pos. | 7.0 | | | 21.6 | 1.64 | | |
| | 231 | Water | 12 | 10.8 | 1.00 | 1.00 | Sl. | 2.5 | | | 15.9 | 1.00 | | |
| Cut tubers, soaked 1 hour, Bliss | 224 | 1.00 | 92 | 9.1 | 1.21 | 1.50 | Sl. | 18.0 | | | 25.6 | | | 5 |
| | 225 | 0.50 | 24 | 9.7 | 1.14 | 1.57 | Sl. | 11.0 | See Note | | 16.4 | | | |
| | 226 | 0.20 | 12 | 9.8 | 1.21 | 1.45 | Pos. | 11.0 | | | 16.0 | | | |
| | 227 | Water | 16 | 9.5 | 1.00 | 1.00 | Neg. | 8.5 | | | 13.8 | | | |
| Cut tubers, soaked 1 hour, Bliss | 177 | 1.00 | 88 | 3.6 | 2.04 | 1.33 | 15.0 | 5.0 | 6.05 | 6.98 | 8.0 | | | 3 |
| | 178 | 0.40 | 76 | 3.1 | 1.20 | 1.50 | Neg. | 3.0 | 5.97 | 6.64 | 3.6 | | | |
| | 179 | 0.16 | 36 | 1.4 | 1.23 | 1.39 | Neg. | 2.5 | 5.97 | 6.51 | 3.3 | | | |
| | 180 | 0.06 | 0 | 0.7 | 0.69 | 1.08 | Neg. | 2.5 | 5.93 | 6.47 | 3.4 | | | |
| | 181 | Water | 0 | 2.0 | 1.00 | 1.00 | Neg. | 2.5 | 5.88 | 6.47 | 4.6 | | | |
| Cut tubers, soaked 1 hour, Bliss | 162 | 2.00 | 100 | 8.9 | 2.04 | 1.42 | 2.0 | 9.5 | 6.14 | | 16.5 | 2.27 | 1.18 | 4 |
| | 163 | 1.00 | 95 | 10.2 | 1.72 | 1.22 | 1.5 | 4.0 | 6.10 | | 17.9 | 1.82 | 1.14 | |
| | 166 | 0.50 | 100 | 9.2 | 1.39 | 1.37 | Neg. | 3.5 | 6.05 | | 12.4 | 1.44 | 1.08 | |
| | 165 | 0.25 | 45 | 10.2 | 1.53 | 1.20 | Neg. | 3.5 | 6.00 | | 9.7 | 1.81 | 1.00 | |
| | 164 | Water | 20 | 7.3 | 1.00 | 1.00 | Neg. | 2.5 | 5.97 | | 8.7 | 1.00 | 1.00 | |
| Cut tubers, soaked 1 hour, Cobbler | 152 | 2.00 | | 7.8 | 1.52 | 1.38 | Pos. | | | | 23.6 | 2.57 | | 4 |
| | 153 | 0.67 | | 8.8 | 1.76 | 1.35 | Neg. | | | | 15.7 | 1.44 | | |
| | 154 | 0.22 | | 8.0 | 1.55 | 1.02 | Neg. | | | | 10.2 | 1.07 | | |
| | 155 | Water | | 7.8 | 1.00 | 1.00 | Neg. | | | | 7.8 | 1.00 | | |

Note: Tissue for lots 224-231 ground with calcium carbonate for the catalase determination.

* pH measurements made with quinhydrone electrode.

an increased amount of catalase in the press-juice. (2) But the thiocyanate absorbed by the tissue, when pressed out with the juice, interfered with the action of the catalase that had been formed. Whether the thiocyanate was interfering with the catalase within the potato tissue before the juice was pressed out is an important question upon which the present experiments furnish no evidence.

The effect of thiourea treatments upon catalase is shown in table 6. The data from the juice obtained from the seed-pieces after excluding the eye-tissue are shown in column 5 and indicate no differences in catalase activity between treated and checks, and no series of readings corresponding to the concentrations of chemicals used. Furthermore when these juices were subjected to dialysis as was done for the thiocyanate lots (see table 4) no differences between treated and checks were obtained.

The catalase determinations on the extracts from the eye-tissue (column 12), however, show definite increases of treated over checks and a fair agreement between the gradation of catalase values and the series of concentrations of thiourea; but, as compared with either ethylene chlorhydrin or sodium thiocyanate, thiourea had much less effect upon the catalase of the juices.

The direct action of thiourea upon catalase of potato juice is not important in connection with these tests. The addition of thiourea to potato juice in amounts greater than about 150 milligrams per 100 cc. of juice retarded catalase activity; amounts smaller than about 50 milligrams produced no effect. Loevenhart and Kastle (8) found a marked increase in the catalase of hog's liver by the addition of thiourea, but a similar effect upon potato catalase was not observed by us.

Peroxidase

Two different methods were used in determining the peroxidase activity: the purpurogallin method and a modification of the Nadi-oxidase reaction. In the purpurogallin method ten cc. of five per cent pyrogallol, 30 cc. of phosphate buffer at pH 6.5, two cc. of 12 volume H_2O_2 , and the required amount of potato juice or extract were added in centrifuge tubes. The mixture was allowed to stand in a constant temperature room either at 23° C. or at 0° C. according to whether it was more convenient to stop the reaction after a few hours or allow it to continue overnight. When the precipitate of purpurogallin, which formed as a result of the peroxidase action of the juice, became large enough to give a good colorimeter reading, the tubes were centrifuged and the precipitate was dissolved in 95 percent alcohol with the aid of heat, was made up to volume, was filtered, and the amount of purpurogallin was estimated by a colorimetric comparison using the check lot as the standard. Usually either one or two cc. of potato juice and five to ten cc. of extract from the eye-tissue gave suitable concentrations for convenient measurements, and, although the amount used

varied somewhat in different tests, in any one test the amounts of juice used and all other conditions of the procedure were exactly the same for the treated and check lots.

In the Nadi method 25 cc. portions of the paraphenylene diamine-alphanaphthol mixture, made up in a citrate buffer of pH 4.5, were placed in centrifuge tubes with 25 cc. of toluene. Sufficient extract or juice to give a convenient reading (usually 1 cc.) and 5 cc. of Dioxygen diluted 1 : 20 were added. The tubes were stoppered and allowed to stand with frequent shaking until sufficient color developed for a satisfactory colorimetric comparison. The peroxidase of the juice brings about the production of indophenol by interaction of the reagents; the indophenol dissolves in the toluene giving a red color which varies in intensity according to the peroxidase activity of the juice tested. The tubes are centrifuged. The top layers are decanted and compared in a colorimeter, using the check as the standard.

The agreement between the two methods was only fair, and, in some cases, quite poor; this divergence is not surprising, since the enzym not only acted upon different substrates in the two cases, but also at two different hydrogen-ion concentrations. But, in general, the principal conclusions that could be reached by a consideration of the data were the same by either method.

The effect of the chemical treatments upon the peroxidase of the press-juice is shown for the ethylene chlorhydrin treatments in table 1, for sodium thiocyanate in table 3, and for thiourea in table 6. In the case of the peroxidase it appears unnecessary to discuss the three chemicals separately as was done for catalase, since the chemicals gave more nearly equal results. The data show in general an increase of the treated over the check, the most favorable concentrations of chemicals giving an increase of about 50 to 100 percent. The relationship between the concentration of the chemical and the peroxidase ratio, however, is not such as to give gradations which correspond exactly to the concentrations of chemical used in the treatment; the agreement is fair but there are several irregularities.

None of the three chemicals had an accelerating effect upon the peroxidase when added directly to potato juice. Ethylene chlorhydrin could be added up to about one percent of the potato juice (by volume) without either increasing or decreasing the peroxidase activity. Sodium thiocyanate at about 50 milligrams per 100 cc. of juice retarded, as did also thiourea at about 200 milligrams, but in the case of neither chemical did lower concentrations cause a detectable increase in peroxidase as measured by the purpurogallin method.

Time Relations in the Development of Catalase and Peroxidase Activity

In order to determine approximately how soon after treatment the increases in catalase and peroxidase began, samples of treated and check

potatoes were taken at intervals of 16, 24, 48, etc. hours after treatment. The results for the ethylene chlorhydrin treatments are shown in table 7 and for sodium thiocyanate and thiourea in table 8. In the chlorhydrin treatments it is seen that the catalase increase is very marked between the 24th and 48th hour and increases as early as the 16th hour after treatment were observed. The increases were greater in the extracts from the eye-tissue than in juices from the not-at-eye portion.

TABLE 7. *Time Relations in Effect of Ethylene Chlorhydrin Treatment of Dormant Potatoes*

| Lot No. | Variety | Hours After End of Treatment | Juice from Tissue Exclusive of Eye-Tissue | | | Juice from Eye-Tissue Only | | |
|-------------|---------|------------------------------|---|-------|--------------------------------|--|-------|--------------------------------|
| | | | Catalase, cc. O ₂ in 1 Min. | | Peroxidase Ratio. Check = 1.00 | Catalase, cc. O ₂ in 1 Min. | | Peroxidase Ratio. Check = 1.00 |
| | | | Treatment | Check | | Treatment | Check | |
| 187 and 188 | Bliss | 24 | 10.1 | 16.0 | 1.07 | 4.9 | 8.2 | 1.82 |
| | | 48 | 16.9 | 11.0 | 2.05 | 24.9 | 5.5 | 2.17 |
| | | 72 | 24.5 | 12.2 | 2.04 | 24.3 | 6.4 | 3.45 |
| | | 96 | 22.2 | 11.3 | 2.20 | 24.6 | 7.0 | 6.12 |
| 191 and 192 | Cobbler | 24 | 9.1 | 7.9 | 1.28 | 10.7 | 9.3 | 1.63 |
| | | 48 | 15.9 | 9.0 | 1.74 | 29.8 | 11.3 | 2.50 |
| | | 72 | 21.2 | 8.6 | 2.68 | 28.7 | 12.2 | 2.52 |
| 211 and 212 | Cobbler | 30 | 10.2 | 5.5 | | 6.5 | 3.5 | |
| | | 56 | 15.1 | 7.5 | | 21.5 | 5.3 | |
| | | 80 | 13.1 | 4.3 | | 25.1 | 6.1 | |
| 213 and 214 | Bliss | 30 | 6.5 | 5.7 | | 8.6 | 2.2 | |
| | | 56 | 14.3 | 7.5 | | 20.5 | 3.6 | |
| | | 80 | 14.8 | 5.5 | | 29.8 | 5.7 | |
| 215 and 216 | Cobbler | 16 | 4.4 | 1.7 | 2.20 | 4.9 | 3.0 | 1.77 |
| | | 23 | 7.6 | 3.8 | 1.98 | 4.0 | 3.3 | 1.11 |
| | | 42 | 15.7 | 6.8 | 2.38 | 15.0 | 4.6 | 1.45 |
| 219 and 220 | Cobbler | 0 | 3.7 | 3.7 | 1.01 | 4.5 | 4.8 | 1.23 |
| | | 16 | 9.3 | 5.8 | 1.96 | 5.6 | 2.3 | 1.23 |
| | | 24 | 9.0 | 4.9 | 1.64 | 6.7 | 3.6 | 1.32 |
| | | 40 | 18.7 | 4.1 | 2.86 | 25.4 | 5.6 | 2.02 |

The peroxidase increases developed in a manner similar to that of the catalase, and although the course of development of peroxidase was not as consistent in the different lots as was the catalase, there was some evidence that the peroxidase increase began even earlier than that of the catalase.

The results with thiourea (table 8) show no consistent increase with respect to time in catalase in the juice from the tissue-not-at-eye, but in the extract from the eye-tissue there is a gradual increase beginning about the 24th to 48th hour; this is true also of the peroxidase, except that the increase in the eye-tissue samples is not gradual but rather uneven. The sodium thiocyanate data on this point (table 8) are in agreement with

TABLE 8. *Time Relations in Effect of Sodium Thiocyanate and Thiourea Treatment of Dormant Potatoes*

| Lot No. | Treatment | Hours After End of Treatment | Juice from Tissue Exclusive of Eye-Tissue | | | | | Juice from Eye-Tissue Only | | | | |
|-------------|------------------------------------|------------------------------|---|-----------------|-------|--------------------------------|-----------------|--|-----------------|-------|--------------------------------|-----------------|
| | | | Catalase, cc. O ₂ in 1 Min. | | | Peroxidase Ratio. Check = 1.00 | | Catalase, cc. O ₂ in 1 Min. | | | Peroxidase Ratio. Check = 1.00 | |
| | | | Thiourea Treatment | NaSCN Treatment | Check | Thiourea Treatment | NaSCN Treatment | Thiourea Treatment | NaSCN Treatment | Check | Thiourea Treatment | NaSCN Treatment |
| 221 to 223 | Cut tubers, soaked 1 hour, Cobbler | 0 | 3.6 | 4.2 | 4.8 | 0.93 | 1.17 | 4.2 | 3.6 | 3.9 | 0.99 | 1.14 |
| | | 24 | 6.3 | 4.5 | 6.2 | 1.01 | 1.35 | 6.9 | 4.6 | 4.8 | 1.12 | 0.93 |
| | | 48 | 8.7 | 3.7 | 8.2 | 1.21 | 1.17 | 8.6 | 8.2 | 7.3 | 1.52 | 1.26 |
| | | 72 | 7.7 | 5.4 | 7.4 | 0.95 | 0.94 | 8.6 | 8.3 | 6.8 | 1.36 | 1.23 |
| | | 96 | 7.6 | 4.9 | 6.7 | 1.21 | 1.29 | 8.2 | 6.2 | 5.8 | 1.33 | 1.33 |
| | | 120 | 8.5 | 6.3 | 7.2 | 0.95 | 1.11 | 12.8 | 8.7 | 7.1 | 2.27 | 1.38 |
| | | 120 | | | | | | | | | | |
| 189 and 190 | Cut tubers, soaked 1 hour, Bliss | 0 | | | | | 0.70 | | | | | |
| | | 24 | | | | | 1.16 | | | | | 1.04 |
| | | 48 | | 6.3 | 11.9 | | 1.26 | | 5.6 | 9.1 | | 1.28 |
| | | 72 | | 4.9 | 8.8 | | 1.42 | | 6.1 | 11.4 | | 1.99 |
| | | 96 | | 4.7 | 7.7 | | 1.61 | | 3.6 | 8.1 | | 1.90 |
| | | 120 | | 5.5 | 7.8 | | 1.43 | | 11.2 | 11.2 | | 2.03 |
| | | 148 | | 10.7 | 11.6 | | 1.56 | | 15.0 | 7.2 | | 3.34 |

those in table 3 in showing no catalase increases in the juice from the not-at-eye tissue at any time during the period of sampling; but, as described in a previous paragraph, this is no doubt due to the inhibiting effect on the catalase resulting from the presence of thiocyanate in the press-juice. It will be remembered that after dialysis of such juices to remove the NaSCN the treated juices were then higher than the corresponding checks. In the eye-tissue samples also, there was no increase in catalase, at least not until after 120 hours. The peroxidase measurements with the exception of one lot show increases beginning about the 72nd or 96th hour.

Reducing Properties of Juices

Methylene Blue Reduction

The solution of methylene blue contained 50 milligrams per liter. To five cc. of the freshly-pressed juice in a narrow test tube one to two cc. of the methylene blue solution were added and the two were mixed. The time required for complete decoloration of the liquid at the bottom of the tube was noted. In cases where the reduction was not complete but only partial, the designations *Sl.* (= slight) and *Pos.* (= positive) were used to distinguish them from the lots called *Neg.* (= negative) in which no reduction at all could be noted at the end of 30 minutes.

The effect of treatment with different concentrations of ethylene chlorhydrin upon the methylene blue reducing capacity of potato juice is shown in table 1, column 8. Under the conditions of the test, juices from the check lots were not able to reduce methylene blue within 30 minutes. The lots treated with favorable concentrations of ethylene chlorhydrin, however, caused reduction within about a minute or less.

Treatments with sodium thiocyanate and thiourea gave juices with much less capacity to reduce methylene blue, as shown in table 3, column 8 and table 6, column 8. In these cases the reduction was greater with the treated than with the checks, but the differences were not as great as with the chlorhydrin treatments.

The pH value of the solution in which the methylene blue reduction is carried out is of importance, the less acid the solution the more rapid the rate of reduction. This was taken into account and it was found that when the treated and check juices were adjusted to the same pH the reduction was always more rapid in the treated lot; furthermore, while check juices were unable to cause reduction at pH values more acid than about 6.20, chlorhydrin-treated juices made acid to 5.75 could still reduce the methylene blue within the 30 minute period. The addition of small quantities of ethylene chlorhydrin, sodium thiocyanate, or thiourea to potato juice did not increase the rate of reduction of methylene blue. Sodium thiocyanate at about 20 milligrams per 100 cc. of juice appeared to retard the action somewhat.

Indophenol Reduction

The indophenol solution was prepared by dissolving 100 milligrams of indophenol in 25 cc. of boiling alcohol and diluting rapidly with water to one liter. When five cc. of potato juice are placed in a test tube and the indophenol solution is run in from a burette, the blue color is at first instantly reduced to a colorless condition; upon the continuous addition of the dye a point is reached at which the blue color is not discharged. The amounts of indophenol decolorized by juices from the potatoes treated with different concentrations of chemicals are shown in tables 1, 3, and 6. It is seen that, while juices from untreated lots absorb about 2.5 cc. of indophenol under the conditions of the test, the juices from the lots treated with favorable concentrations of chemicals absorb 5 to 36 cc. In all tests but one there was a good correlation between the concentration of chemical used in treating the potato and the indophenol absorption of the press-juice. The order of effectiveness of the three chemicals is first, ethylene chlorhydrin, then sodium thiocyanate, and finally thiourea; but even in the case of thiourea the value for the treated is about twice that of the check. The indophenol absorption of juices obtained from the whole tuber treatments with ethylene chlorhydrin was especially high.

It should be emphasized that this reaction can be obtained when boiled juice is used, and, therefore, differs to a certain extent from the methylene blue reduction which is enzymatic, and does not occur in boiled juice.

Self-reducing Properties

When potato juice is allowed to come in contact with air it becomes first reddish, and later brown, or even nearly black. In these experiments, during the process of extraction the juices were exposed to the air and became more or less dark brown. When these juices were poured into test tubes, they gradually became lighter in color, and finally nearly canary yellow. One of the most distinct results of the chemical treatments was to favor the rapidity of this self-reduction; and, furthermore, the order in which the juices became decolorized was in the order of the series of concentrations of chemicals used in treating the potatoes. With all three chemicals it was observed that the juice from the highest concentration of chemical became yellow in the shortest time, and then followed in order the different treatments, the check lot remaining brown for the longest time. The naturally-occurring pigment-forming system, therefore, was a better indicator than methylene blue for distinguishing between different concentrations of chemicals in their effect upon the reducing properties of juices. Also, the three chemicals were more nearly alike with respect to this self-reduction than they were toward methylene blue reduction. Thus, in the thiourea treatments the differences between treated and check were not large, as shown in table 6; but the capacity of thiourea treatments to give quicker reduction of the color of oxidized juices was very distinct, and

gave gradations with respect to these characteristics corresponding closely to the graded series of concentrations of chemicals.

It is likely that the capacity of dark colored juices from thiourea-treated potatoes to decolorize when allowed to stand is connected with the ability of thiourea to retard darkening of juices or tissue when the thiourea is added directly to the juice, or when pieces of potato tissue are dipped into solutions of thiourea and allowed to stand in air. In such cases the darkening can be completely inhibited if the proper concentration is chosen.

Iodine Reduction

When ten cc. of ten percent trichloroacetic acid are added to five cc. of potato juice the mixture will absorb appreciable amounts of 0.01 *N* iodine. Using starch as an indicator of an excess of iodine, it was found

TABLE 9. *Reducing Properties of Juice From Potatoes Treated With Chemicals*

| Chemical Treatment | Lot No. | Conc. of Chemical Used | 0.01 <i>N</i> Iodine Absorbed cc. | Reduction of Phosphotungstic Reagent. Ratio Treated Check |
|---|---------|------------------------|-----------------------------------|---|
| NaSCN, cut tubers, soaked 1 hour | 167 | 1.00% | 1.55 | 2.02 |
| | 168 | 0.50% | 1.25 | 1.54 |
| | 169 | 0.25% | 1.00 | 1.48 |
| | 170 | 0.13% | 0.75 | 0.87 |
| | 171 | Water | 0.70 | 1.00 |
| NaSCN, cut tubers, soaked 1 hour | 236 | 1.00% | 1.65 | 1.95 |
| | 237 | 0.50% | 1.50 | 1.78 |
| | 238 | 0.25% | 1.05 | 1.38 |
| | 239 | Water | 0.85 | 1.00 |
| Chlorhydrin, whole tubers, vapor method | 137 | 1.00 cc. | 2.75 | |
| | 138 | 0.20 cc. | 1.15 | |
| | 139 | 0.04 cc. | 0.30 | |
| | 140 | 0.008 cc. | 0.20 | |
| | 141 | Check | 0.20 | |
| Chlorhydrin, whole tubers, vapor method | 193 | 1.00 cc. | 2.10 | 1.79 |
| | 194 | 0.25 cc. | 1.00 | 0.98 |
| | 195 | 0.06 cc. | 1.15 | 1.00 |
| | 196 | Check | 0.85 | 1.00 |
| Chlorhydrin, cut tubers, dip method | 207 | 45 cc. | 0.75 | |
| | 208 | 15 cc. | 0.85 | |
| | 209 | 5 cc. | 0.75 | |
| | 210 | Water | 0.45 | |
| Chlorhydrin, cut tubers, dip method | 187 | 30 cc. | 2.00* | |
| | and | Check | 1.05 | |
| | 188 | 30 cc. Check | 3.30 0.90 | |
| Thiourea, cut tubers, soaked 1 hour | 162 | 2.00% | | 2.36 |
| | 163 | 1.00% | | 1.13 |
| | 165 | 0.50% | | Lost |
| | 166 | 0.25% | | 1.13 |
| | 164 | Water | | 1.00 |

*First pair of readings 48 hours, and second pair 72 hours after treatment.

that juices from the potatoes that had been treated with ethylene chlorhydrin and sodium thiocyanate absorbed larger quantities of iodine than juice from untreated potatoes. The results are shown in table 9. The thiocyanate-treated lots absorbed about twice as much iodine as the checks, and for the ethylene chlorhydrin treatments the divergence was even greater. The amounts of iodine absorbed by the juices formed in the different tests a series of values which corresponded fairly well with the amounts of chemicals used in treating the potatoes. Sodium thiocyanate itself will absorb iodine in neutral or alkaline solutions, but not in the acidity produced by the amounts of trichloroacetic acid added in making the tests.

It will be noted that no results with thiourea are shown. This is because thiourea itself absorbs iodine in acid solution, hence although this test was made, and, although the juices from the treated lots absorbed more iodine than the checks, it seemed that the procedure was more nearly a measure of the amount of thiourea absorbed by the potato than of the substances for which the iodine test was being used.

Titration with 0.01 *N* iodine in acid solution, using nitroprusside as an indicator, has been recommended as a measure of glutathione (see Tunnicliffe, 12). Starch has also been tried as an indicator, but generally regarded as unsatisfactory, merely because it gives higher results than nitroprusside. It has, however, been recommended by Blanchetiere and Melon (1). Potato juice gives a very feeble nitroprusside reaction and therefore nitroprusside can not be used as an indicator. It is probable that the substance responsible for the iodine reaction in the potato is not glutathione, but resembles the reducing substance described by Szent-Györgyi (10).

Phosphotungstic Reagent Reduction

If to two cc. of Folin's (6) improved uric acid reagent (free from the phenol reagent) are added two cc. of boiled filtered potato juice, together with ten cc. of 20 percent sodium carbonate, a blue color will develop within a few minutes because of the reduction of the tungstic reagent. It was found that the intensity of this blue color was greater with juices from the potatoes that had been treated with chemicals than with the check lots, and that a colorimetric comparison of treated and check could be made. The results are shown in table 9 from which it is seen that the reducing action of the juice from potatoes receiving favorable amounts of chemical was approximately twice that of the check juices.

Effect of Method of Extraction, Time of Standing, and Aëration Upon Reducing Properties

The reducing capacity of a juice decreased to low values when the juice was exposed to air in a thin layer. Under these conditions its capacity to reduce methylene blue was completely lost, and its effectiveness in

TABLE 10. *Relation of Reducing Properties to the Method of Extraction and Time of Standing of Juice*

| Lot No. | Treatment | Cubic Centimeters of 0.01 N Iodine Absorbed | | | | | | Indophenol Reduced cc. | | Reduction* of Phosphotungstic Reagent | | Reduction of Methylene Blue, min. |
|---------|--|---|-------------|-------------------------|-------------|--|---------------------------------|------------------------|---------------------------------|---------------------------------------|--|-----------------------------------|
| | | Amount of Chemical | Press-juice | | | Trichlor-acetic Acid Extract of Tissue | Boiling Water Extract of Tissue | Fresh Juice | Boiling Water Extract of Tissue | Juice | Trichlor-acetic Acid Extract of Tissue | Fresh Juice |
| | | | At Once | Stood $\frac{1}{2}$ hr. | Stood 1 hr. | | | | | | | |
| 197 | Ethylene chlorhydrin, whole tubers, vapor method | 1 cc. | 2.50 | 3.10 | 3.35 | 3.10 | 2.65 | 18 | 22 | 2.00 | 1.16 | 1/4 min. |
| 198 | Ethylene chlorhydrin, whole tubers, vapor method | 1/4 cc. | 2.10 | 2.45 | 2.55 | 3.00 | 2.70 | 13 | 22 | 1.51 | 1.18 | 3 min. |
| 199 | Ethylene chlorhydrin, whole tubers, vapor method | 1/16 cc. | 1.10 | 1.15 | 1.15 | 2.40 | 2.25 | 6 | 21 | 0.70 | 0.93 | Not complete after 30 min. |
| 200 | Not treated | Check | 1.50 | 1.80 | 1.55 | 2.65 | 2.20 | 4 | 21 | 1.00 | 1.00 | Not complete after 30 min. |

* Colorimetric comparison with check at 1.00 as standard.

reducing iodine and indophenol was much lower. In addition, the reducing property of a juice was found to depend upon whether the tests were applied upon press-juice or upon extracts obtained with boiling water or with trichloracetic acid. The results of a series of tests on the behavior of the reducing capacity are shown in table 10.

The following observations are to be considered: (1) In treated juices the power to reduce 0.01 *N* iodine increased on standing. (2) Treated juices reduced methylene blue and this action was inhibited by boiling. (3) The reducing action of the juice on indophenol and 0.01 *N* iodine disappeared rapidly on aërating the fresh juice, but not the boiled juice. (4) Extracts prepared by dropping the tissue into boiling water, or extracting with trichloracetic acid, show much smaller differences when titrated with 0.01 *N* iodine than the expressed juice. These results would be in agreement with the assumption that the substance or substances responsible for the indophenol, iodine, and phosphotungstic reduction are present in the tissue in the reduced form; that these are partially oxidized in the process of extraction, but reduced back again in the treated juice.

Amylase and Invertase

The activity of both these enzymes may be measured by their effect in causing an increase in reducing sugar by the splitting of added substrates. But the measurements are difficult with potato juice for the reason that the amylase activity is not high and the correction due to the sugar in the blank test is relatively high in comparison with the increase it is desired to measure. Invertase may be studied simultaneously but, to make a comparison between treated and check juices in our experiments, it was necessary to add cane sugar in excess to the juice, since juices from lots treated with chemicals contained initially higher amounts of cane sugar than the check lots.

To estimate the amylase and invertase activity of a given juice at least four sugar determinations (eight or twelve including duplicates) were needed for each lot of juice. Since there were usually four or more lots in each experiment, the time required for performing the necessary operations made it impossible to carry out more than preliminary determinations of amylase and invertase measurements. The results of such tests are shown in tables 11 and 12. It is seen (columns 7 and 8 in table 11) that the amylase and invertase activities of the treated lots were higher than the check, and that a series of graded values was obtained corresponding well with the gradation of chemical concentrations used in treating the potatoes. Column 6 shows that the sucrose values were higher in the treated than in the check. But from column 3 it will be seen that the pH values were also different in the treated and check lots, and it was necessary to take this into account in another experiment. In order to determine the effect of the pH value of the juice upon amylase and invertase activity, a portion of the juice from the treated lot was adjusted to the pH of the

check lot by the addition of acid, and the pH of a portion of the check lot was adjusted to that of the treated lot by the addition of alkali. The amylase and invertase activity of the four lots were then determined and the results are shown in table 12. It is clear that the difference in pH can not account for the difference in enzym action between treated and

TABLE 11. *Effect of Ethylene Chlorhydrin Treatment Upon Amylase and Invertase of Potato*

| Treatment | Conc. of Chem. cc. per l. | pH | Potassium Permanganate Values* Resulting from Sugar Determination. cc. N/20 KMNO ₄ per 5 cc. of Juice | | | | |
|---|---------------------------|------|--|----------------------------|------------------|-----------------|-------------------|
| | | | Boiled Juice | Juice Increase on Standing | Sucrose† Present | Amylase Values‡ | Invertase Values§ |
| Chlorhydrin, whole tubers, vapor method | 1.00 | 6.73 | 0.00 | 2.30 | 22.15 | 22.25 | 11.23 |
| | 0.33 | 6.47 | 0.17 | 4.16 | 21.38 | 21.44 | 9.09 |
| | 0.11 | 6.47 | Trace | 3.78 | 18.40 | 20.40 | 7.90 |
| | 0.09 | 6.51 | 0.93 | 3.39 | 15.32 | 19.36 | 6.16 |
| | Check | 6.34 | 2.92 | 1.18 | 13.53 | 16.63 | 6.10 |

* Averages of three duplicate determinations.

† Representing the relative amounts of sucrose in the juices.

‡ Representing the increase in reducing power due to hydrolysis of added starch after making correction for blanks.

§ Represent the increase in reducing power due to inversion of added sucrose after making correction for blanks.

TABLE 12. *Effect of pH on the Comparison of Treated and Check Lots With Respect to Amylase and Invertase*

| Treatment | | Potassium Permanganate Values Resulting from Sugar Determinations. cc. N/20 KMNO ₄ per 5 cc. Juice | | | | |
|--|---|---|----------------------------|-----------------|----------------|------------------|
| | | Boiled Juice | Juice Increase on Standing | Sucrose Present | Amylase Values | Invertase Values |
| Chlorhydrin, whole tuber, treatment 1 cc. per l. 24 hrs. | Juice as pressed pH = 6.8 | 0.15 | 1.80 | 22.45 | 20.33 | 7.13 |
| | Juice adjusted to pH of check lot = 6.3 | 0.16 | 1.30 | 24.34 | 16.20 | 5.07 |
| Check, not treated | Juice as pressed pH = 6.3 | 6.40 | 1.61 | 15.2 | 7.07 | 1.90 |
| | Juice adjusted to pH of treated lot = 6.8 | 6.33 | 1.77 | 14.3 | 15.55 | 3.35 |

Note: See foot-notes to table 11 which apply here also.

check since even at the same pH the treated was 50 to 100 percent higher than the check.

It is realized that further work is needed before conclusions can be drawn, and in particular these experiments, which were carried out with

ethylene chlorhydrin, should be extended to other chemicals. The effect upon amylase of the chemicals themselves when added to potato juice is now being investigated, especially with regard to sodium thiocyanate, because of the previous work of Johnson and Wormall (7) which indicated a direct stimulative effect of this chemical upon the amylase of the saliva and potato juice, at any rate upon the dialyzed enzym.

Acetaldehyde Formation

Boresch (2), who investigated the effect of the warm-bath treatment of twigs of woody plants in breaking dormancy, found increased amounts of acetaldehyde in treated tissue, and he believed that the aldehyde formed was an effective agent in inducing growth of buds. We made some preliminary measurements of acetaldehyde in treated and check lots of potatoes, first steam distilling the tissue to separate the acetaldehyde, and then using the method of Tomoda (11). The test can not be regarded as specific for acetaldehyde since it is given by any aldehyde or ketone. The aldehyde measurement by this method can be expressed in terms of cubic centimeters of 0.1 *N* iodine. Only small amounts of acetaldehyde were found, but in the three tests made the ethylene chlorhydrin treated potatoes gave higher values than the check lots. Thus, using 250 grams of minced tissue for each test, the titration values were: treated 0.85 cc., check 0.25 cc.; treated 0.45 cc., check 0.30 cc.; treated 0.60 cc., check 0.30 cc. The effect of sodium thiocyanate and thiourea treatments upon the development of acetaldehyde in potatoes was not determined.

Because of the low values obtainable from even such a large amount of tissue, it was concluded that neither time nor tissue could be sacrificed for further tests of acetaldehyde at that time.

But even if it could be demonstrated that larger amounts of acetaldehyde are formed in treated tissue, it could not be concluded from this that acetaldehyde was the cause of the breaking of dormancy. Since acetaldehyde is an intermediate product in respiration, and since Smith (9) has shown that the ethylene chlorhydrin treatment greatly increases the respiration of potato tubers, a more likely explanation is that the acetaldehyde is a result of the increased life activity, and not a cause of its initiation. In other words the dormancy has been broken and considerable cell activity has occurred before the increase in acetaldehyde starts to take place.

Effect of Presence and Absence of Eyes in the Chemical Treatment of Potato Tubers

To determine whether the changes that occur when the cut tubers are treated with chemicals also take place in the absence of eyes, the eyes were removed from a portion of the seed-piece by reaming out with a knife blade. The two lots (with and without eyes) were then subjected to the same chemical treatment, and after several days were compared with each

TABLE 13. *Effect of Presence and Absence of Eyes in the Chemical Treatment of Dormant Potatoes*

| Lot No. | Treatment | Description | Catalase, cc. O ₂ in 1 min. | | Peroxidase | | | | Methylene Blue Reduction Min. | | Indophenol cc. Absorbed | | 0.01 N Iodine Absorbed | |
|------------------|---|-----------------------------|---|-------|---------------|-------|-------------|-------|-------------------------------------|-------|----------------------------|-------|---------------------------|-------|
| | | | | | Purpurogallin | | Nadi method | | | | | | | |
| | | | Treated | Check | Treated | Check | Treated | Check | Treated | Check | Treated | Check | Treated | Check |
| 148 to 151 | Cut tubers, soaked 1 hour in 1 percent NaSCN, Cobbler | Seed pieces with eyes | 13.0 | 5.1 | 2.66 | 1.00 | 1.51 | 1.00 | Sl. | Neg. | 6.0 | 3.5 | 0.35 | 0.25 |
| | | Seed pieces without eyes | 12.8 | 8.8 | 3.12 | 2.56 | 1.94 | 1.26 | Pos. | Pos. | 5.0 | 4.0 | 0.45 | 0.40 |
| 201 to 204 | Ethylene chlorhydrin, cut tubers, dip meth- od, 50 cc. per l., Cobbler | Seed pieces with eyes | 19.5 | 7.5 | 4.45 | 1.00 | 1.29 | 1.00 | 0.25 | Neg. | 36.0 | 2.0 | 4.30 | 0.65 |
| | | Seed pieces without eyes | 17.5 | 12.9 | 3.74 | 2.23 | 1.37 | 1.04 | 0.25 | Neg. | 37.0 | 2.5 | 4.25 | 0.75 |

other and with two other lots, with and without eyes, which received no treatment at all. The results are shown in table 13. The same changes that were induced in the lots having eyes were also found to have occurred in the lots without eyes. The differences between treated and check were not as great when the eyes were absent for the reason that the check lots without eyes gave in general higher readings than check lots with eyes. This may be due to the larger amount of cut surface which was exposed by the removal of the eye from the seed-piece, or to a wound effect from cutting, or both.

Relation of Percentage of Sprouting to the Results of the Tests on the Potato Juices

In tables 1, 3, and 6, column 4 are given the percentage germination of the lots receiving the various chemical treatments. In the sense that the gradation of percentage germinations corresponded both to the series of chemical treatments and to the results of the enzym tests made upon the juices obtained from the potatoes, it may be said that a general relation was found. Certainly the most favorable concentrations of chemicals for inducing sprouting also gave the largest values in catalase, peroxidase, and other properties tested for. But the correlation can not be regarded as a very close one when the results are examined in detail. Thus, the thiourea responses were only small in many respects and practically zero for the catalase in the tissue exclusive of eyes, and yet the sprouting response was good. The failure of the sodium thiocyanate and thiourea treatments to furnish juices with the high capacity to reduce methylene blue such as was characteristic of the ethylene chlorhydrin treatments, when viewed in relation to the evident ability of these treatments to induce favorable sprouting, indicates that no close quantitative connection exists between methylene blue reduction capacity and sprouting. Even in the case of the ethylene chlorhydrin treatments, the whole-tuber treatments showed greater differences between treated and check lots than the cut-tuber treatments did, but the whole-tuber treatments were less effective in inducing germination. No single test could be used as an indicator of the capacity of a given lot of potatoes to sprout when planted; it is only when sprouting response is compared with the tests as a whole, that the general relation between increased enzym activity and high percentage sprouting becomes evident.

DISCUSSION

It is important to note that the increases in enzym activity that were found to result from the treatment of potatoes with chemicals were not direct effects of the chemicals upon the enzymes themselves. In no case were any of the chemicals capable of increasing appreciably the enzym activity of the press-juice; they could cause depression if the concentration of the added chemical was high enough, but within the range of concentra-

tion at which the chemical could likely exist in the juice, no increase in activity was observed. So far as these enzymes were concerned the chemicals did not act by stimulating enzyme action; it would be better to say that they induced the living matter to produce larger amounts of (or more active) enzymes.

An interesting feature of the experiments is the result showing that chemical treatment increased the enzyme activity of potato tissue containing no buds. The tissue in these cases consisted largely of pith cells which are incapable of further growth except to form cork layers after injury by cutting or bruising. And yet these cells responded readily to the chemical treatments as is shown by the increased enzyme action of the press-juice obtained from them. It is true that, on the whole, larger changes and quicker responses were found in the eye-tissue, but to what extent the changes in the tissue at some distance from the bud influenced the enzyme activity at the eye or even the capacity of the bud to start into growth is not shown by these experiments, and is a problem that needs further work.

The effect of the ethylene chlorhydrin treatments in causing a change in the pH value of the juices (in the direction of reduced acidity) was very striking, especially when the whole tubers were exposed to the vapors of the chemical. On letting the tubers stand several days the acidity shift amounted in some cases to approximately a whole pH unit which is a ten-fold change in hydrogen-ion concentration. In the dip-method the shift was about 0.5 of a pH unit. A direct effect of ethylene chlorhydrin itself in causing this change in acidity can not be important here since an aqueous solution of ethylene chlorhydrin is acid, not alkaline, and furthermore the amounts of ethylene chlorhydrin absorbed by the tissue are too small to be effective in altering the pH in either direction. It would be interesting to know what changes within the tissue were responsible for this alteration of the pH. Potato juice is well buffered and the internal changes must have been extensive in order to give this result. Since the pH and buffer value of plant juices are influenced to a large extent by the content of organic acids and phosphates, quantitative measurements of these substances, together with the amounts of soluble cations, are desirable in connection with this problem.

We are aware of the possibility that the pH measurements may have been influenced by the increased reducing capacity of the juice and that the actual change in hydrogen-ion concentration may not have been so great as the data indicate. This point needs further investigation. But in the cases where the electrometric measurements showed a large reduction in acidity merely applying indicators to the surface of the tissue also showed a large shift in the alkaline direction.

We make no claim that the changes in enzyme activity which are here reported are to be looked upon as the causes of the growth of buds or as furnishing proof as to the causes of the previous dormancy. They constitute

the measurements which have been made on the changes of the internal conditions which follow the treatment of the potato with chemicals. They are correlated with the initiation of growth processes and our data represent the result of an effort to push the measurements back to as early a stage of development as possible. It will be remembered that some of the evidence as to the initiation of change reached back to the first 24 hour period after treatment. It is clear, however, that the effect upon the living matter must have been produced at even an earlier hour, that the effect was first upon the living matter which was induced to begin activity, and which then brought about the changes that could be measured.

SUMMARY

1. This is a report of experiments on the enzym activities of juices obtained from dormant potatoes that had been treated with chemicals which break the rest period, the measurements being made after the treatment but before visible sprouts appeared.

2. The effects of three chemicals were studied: ethylene chlorhydrin ($\text{ClCH}_2\text{CH}_2\text{OH}$), sodium thiocyanate (NaSCN), and thiourea (NH_2CSNH_2). Although these three are different in chemical character they all break the dormancy of freshly harvested potatoes.

3. Increases in catalase, peroxidase, and the reducing properties of the juice as measured by the reduction of methylene blue, indophenol, iodine, and phosphotungstic reagents were observed. The increases were more marked in the case of the ethylene chlorhydrin treatments than for the other chemicals.

4. Increases in catalase and peroxidase began within about 24 hours after the end of the treatment with ethylene chlorhydrin, but the response to sodium thiocyanate and thiourea was less marked and occurred less quickly.

5. The increases in enzym activity were not direct effects of the chemicals upon the enzymes. In no case could the enzym activity of the press-juice be increased by the addition of the chemical to the juice. The chemical effect was indirect and was brought about by the action of the chemical upon the potato and not upon the enzymes in its juice.

6. In almost all cases when the potatoes were treated with different amounts of a chemical arranged in a descending series with respect to concentration, the juices obtained from these lots at a later period also showed a series of enzym readings corresponding to the series of concentrations of chemicals originally applied to the potatoes. This was not true, however, of the catalase readings in the thiocyanate treatments until after the press-juice had been dialyzed. The juice from thiocyanate-treated potatoes contained appreciable amounts of thiocyanate which, as shown by separate experiments, has a retarding effect upon catalase. A short period of dialysis allowed a separation of the thiocyanate from the enzym,

after which the catalase values of the thiocyanate-treated lots were higher than the checks, and gave a series of readings corresponding to the concentrations of chemicals used in treating the potatoes.

7. Ethylene chlorhydrin treatments induced a change in the pH of the juice in the direction of decreased acidity; and the amount of change in pH was related to the concentration of chemical applied in the treatment of the tissue. Only small changes in pH resulted from the sodium thiocyanate and thiourea treatments.

8. The enzym changes were greater in the eye-tissue, and in most cases started sooner there than in the rest of the seed-piece. But treatments of potato pieces having no eyes showed that the same changes in enzym activity occurred as in pieces containing eyes, the amount of the change being merely somewhat less. Presence of eyes was not necessary in the enzym responses of tissue.

9. There was a general relation between the sprouting response and the enzym measurements, since the treatments which induced good sprouting were also effective in causing the potatoes to furnish a juice of high enzym activity. The correlation between enzym activity and sprouting was not found to be close, however, when the data were examined in detail. Thus, the sodium thiocyanate and thiourea treatments were much less effective in increasing enzym activity than would have been expected on the basis of the favorable sprouting response. And the enzym activity of juices was greater from whole-tuber than from cut-tuber treatments in the case of ethylene chlorhydrin, although the cut-tuber method gives the better response in sprouting.

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THE DEVELOPMENT OF THE ZOÖSPORES IN THE OÖMYCETES AT OPTIMUM TEMPERATURES AND THE CY- TOLOGY OF THEIR ACTIVE STAGES¹

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INTRODUCTION

Any satisfactory study of the cytology of the zoöspores of the Oömycetes requires a sound understanding of what constitutes healthy conditions for their development and activity. Hitherto no means of obtaining perfectly healthy zoöspores in large numbers under optimal conditions had been perfected. Since the optimal conditions of development were determined for each species by a rigorously controlled experimental procedure, the study has two chief aspects: the physiological, dealing with the conditions determining zoöspore production under optimum and other conditions; and the cytological, dealing primarily with the mechanism of cilia formation.

The morphology and especially the ciliation of zoöspores has been of interest to botanists since the earliest microscopic studies of the thallophytes. Several phylogenetic classifications have been constructed around the types of ciliation found in zoöspores of different groups of the fresh water algae and reference has been frequently made in mycological literature to the details of zoöspore formation and ciliation in fungi. In spite of the biological importance of zoöspores and the long-continued interest in them, comparatively little is known concerning significant details in the formation of the cilia, either in the algae or the fungi. With the exception of the investigations of Klebs (27) and Kauffman (25, 26) relatively little is known in genera other than *Saprolegnia* regarding the stimuli which bring about the formation of sporangia and zoöspores and the conditions most favorable for the maximum activity of the latter.

HISTORICAL

In many cases the cilia emerge from a specially differentiated part of the protoplast; the literature dealing with this question is reviewed by the

¹ Paper from the Department of Botany of the University of Michigan no. 321.

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writer (12) in another paper. It is evident that in a number of genera of algae and fungi, the insertions of the cilia are in chromatic basal granules just inside the plasma membrane. These basal granules have been identified as blepharoplasts. West and Fritsch (44) have said, however, that the general occurrence of blepharoplasts in motile protophyta is still in doubt.

There have been numerous references in the literature to the temperature factor in so far as it favors zoospore formation in various genera of the Oömycetes. De Bary (3) first reported that in *Cystopus* zoospore formation may occur at any temperature between 5° and 25° C. Klebs (27) has shown that in *Saprolegnia mixta* de Bary, at 1° to 2° C., zoospores are liberated after 48 hours; at 6° to 8° C., zoospores are liberated after 24 hours, while the optimum lies between 24° and 28° C. At the latter temperature zoospores are formed in from 5 to 6 hours while the maximum temperature for this process lies between 32° and 33° C. Melhus (30) has shown that zoospores may be formed by the conidia of *Cystopus candidus* (Pers.) Lév. at temperatures ranging from very near zero to 25° C., and that the optimum is 10° C. A number of investigators have mentioned the temperature-relation for zoospore formation in the genus *Phytophthora*. Melhus (31) points out that there is a very definite temperature-relation for zoospore formation in *Phytophthora infestans* (Mont.) de Bary and that the optimum temperature lies between 12° and 13° C. Rosenbaum (38) who worked with various species of *Phytophthora*, states in his discussion of zoospore formation by the conidia, that "the suspension of spores was . . . held at a temperature of about 15° C." and further that "at the end of from two to five hours a large number of the conidia had germinated." Coker (9) at various places in his discussion of the different species of the Saprolegniaceae, has merely mentioned the temperatures at which certain members of the family form zoospores. In *Saprolegnia anisospora* de Bary, the spores are said by Coker to be liberated in spring water at a temperature of 13° C. The temperature at which zoospores were found to be formed in other genera and species discussed by Coker were as follows: *Isoachlya toruloides* Kauff. & Coker, 26° C.; *I. unisporea* Coker & Couch, 30° C.; *Protoachlya paradoxa* Coker, 26° C.; *Achlya apiculata* de Bary, 22° C.; *A. klebsiana* Pieters, 18–24° C.; *A. orion* Coker & Couch, 21.5° C. In his discussion of *Apodachlya brachynema* (Hildeb.) Prings. he says "The sporangia are very whimsical about opening at room temperature (about 60–70° F.) but rarely fail to discharge their spores if kept in an ice box." Jones and Drechsler (24), working with *Aphanomyces euteiches* Drechsler, reported that "with young thalli at a temperature of about 20° C., evacuation of the sporangial filaments was found to begin about six to seven hours after washing was completed," and have shown that the temperature range for spore formation in this fungus is from 9° to 11° C. to 33° to 35° C. Nishimura (34) has reviewed the literature regarding zoospore formation in the genus *Plasmopara* and great divergence is shown to exist in the data

given by investigators who have studied temperature relations to zoöspore formation in these plants.

MATERIAL AND METHODS

In the writer's work twelve Oömycetes were used: *Allomyces arbuscula* Butler, contributed by Dr. Bessie B. Kanouse of the University of Michigan Herbarium, who obtained it from Dr. W. C. Coker of Chapel Hill, North Carolina, March 6, 1926, where it was isolated from soil under grass; *Apodachlya brachynema* (Hildeb.) Prings., contributed by Dr. Kanouse, who collected it on submerged fruits of apple and of *Crataegus*, Ann Arbor, Michigan; *Rhipidium europaeum* von Minden, collected by Dr. Kanouse on submerged fruit bait exposed in a spring fed pond, March 20, 1929, Ann Arbor, Michigan; *Saprolegnia monoica* var. *glomerata* Tisenhausen, contributed and determined by Dr. Kanouse from a culture collected near Ann Arbor in 1925; *Isoachlya paradoxa* (Coker) Kauffman, was contributed by Dr. Kanouse. It was found near Ann Arbor June 10, 1926, identified by Dr. Kauffman; *Achlya conspicua* Coker, contributed by Dr. Kanouse, collected on a submerged apple in a muddy ditch, March 20, 1929, Ann Arbor, Michigan; *Aphanomyces euteiches* Drechsler, received by Dr. Kanouse² from Dr. C. Drechsler of Washington, D. C.; *Phytophthora cactorum* (Cohn & Lebert) Schroeter, obtained from Professor H. H. Whetzel of Cornell University, isolated from apple August 25, 1920; *P. erythroseptica* Pethybridge, obtained from Professor H. H. Whetzel, October 31, 1920; *P. palmivora* Butler, obtained from Dr. L. H. Leonian, of Morgantown, West Virginia, who obtained it from Butler by way of Pethybridge. Leonian (29) states that the organism was isolated by Ashby from Cacao fruit in the West Indies; *P. terrestris* Sherbakoff, sent by Dr. D. Reddick, November 4, 1920, who obtained it from Dr. Sherbakoff; *Phytophthora* sp., obtained from Dr. D. Reddick of Cornell University, November 4, 1920. This plant was isolated from tomato by Dr. Reddick.

All cultures used were grown from single-spore cultures isolated by the method developed by Kauffman (25). With the exception of cultures of the genera *Phytophthora* and *Allomyces*, they were grown on sterile pea broth for 24 to 48 hours. Young vigorous cultures were used in all cases.

Subcultures were made by using sterile pea broth and adding a very small portion of mycelium taken from a similar culture not more than four days old. So-called conductivity water³ was used in all cultures. The mycelium actually used in the experiments was taken from a vigorous culture which was 24 or 48 hours old which had been grown either at room

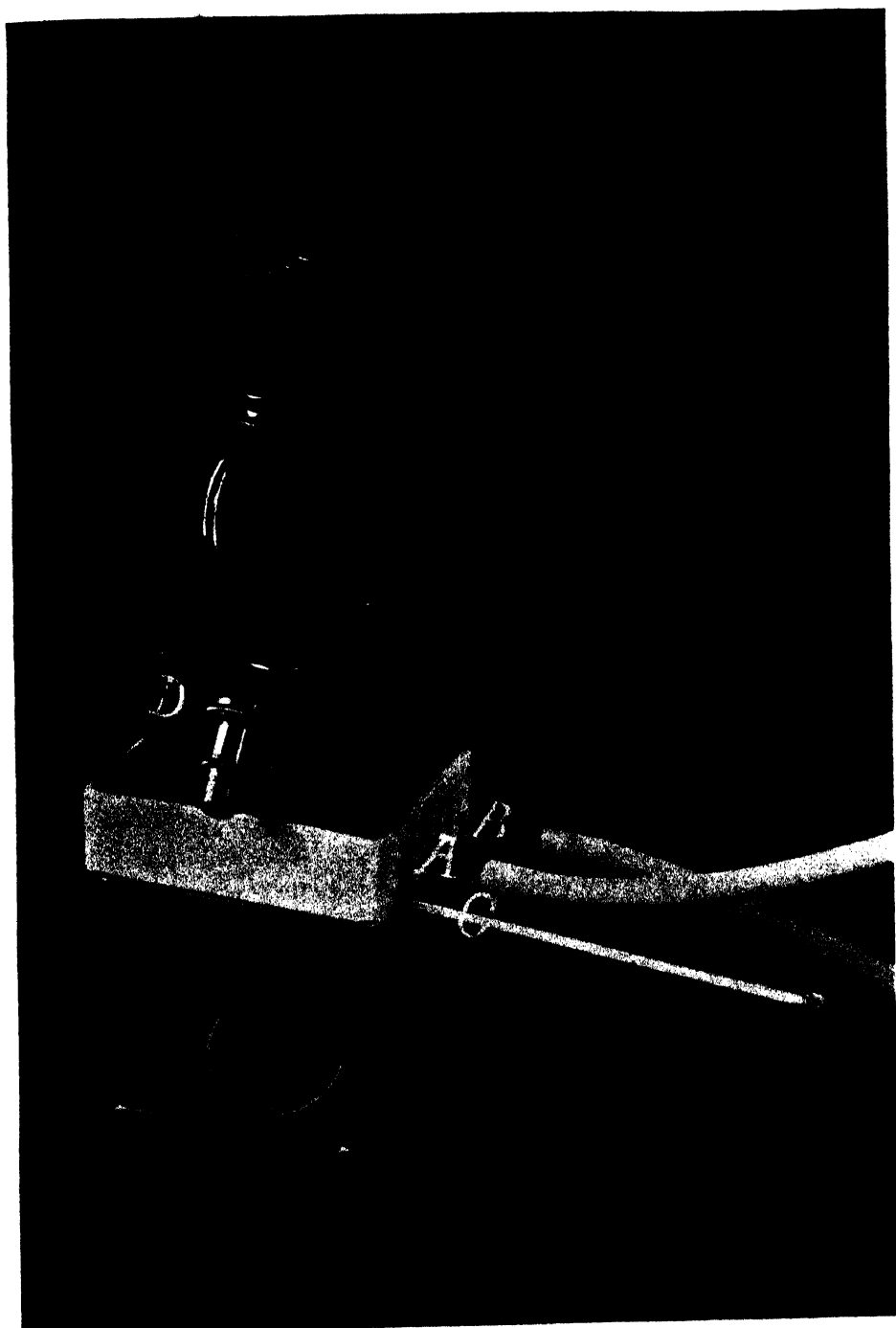
² For providing the many cultures and for numerous helpful suggestions thankful acknowledgment is hereby made by the writer to Dr. Kanouse.

³ The best results were obtained by the use of water free from the minute trace of metallic ions which occurs in most ordinary distilled water. It was possible to obtain from the chemical laboratory water redistilled in quartz, used for electrical conductivity measurements.

temperature (usually between 20° and 22° C.), or in a cold room at a temperature varying between 10° and 12° C. It was necessary to allow the cultures in the cold to grow at least one additional day before sufficient mycelium had developed. It was found that for the major part of the experiments the cultures in stock pea broth which had been grown at temperatures varying between 20° and 22° C. were the most satisfactory, since the most uniform results were obtained from mycelium which had grown rapidly and was in a vigorous condition.

Cultures of *Allomyces arbuscula* were grown on sterile flies in sterile conductivity water at 26° C. until a good development of mycelium was obtained. The mycelium was used before there was any indication of developing sporangia or oögonia. The mycelium from the young culture in pea broth, in the case of species of *Achlya*, *Aphanomyces*, *Isoachlya*, and *Saprolegnia*, and from the young culture on the fly in the case of *Allomyces arbuscula*, was removed either by cutting out a portion with sterile scissors or by tearing out one piece with a sterile platinum hook. This young vigorous mycelium was washed for one hour through six changes of sterile conductivity water, then mounted in hanging drops of well-aërated sterile conductivity water. The preparations were kept in *micro-stage constant temperature chambers* which were placed on the stages of a row of microscopes each under a 16-mm. objective for continued observation during the considerable time required for the development of the sporangia and the liberation of the zoöspores. When a comparable series of experiments was being run in these chambers to determine optimum temperatures, lights for all of the microscopes in use were turned on and off at the same time in order to have all conditions excepting temperature exactly alike for the entire series.

In the experiments dealing with the effect of temperature on the development of sporangia and the formation of zoöspores, warm and cold micro-stages were used for constant temperature chambers. The warm stage used was the micro-slide incubator for electric heating, manufactured by the Chicago Surgical Equipment Company. This instrument is adjustable for temperatures from room temperature to slightly above 40° C. and permits of exact control for all of the higher temperatures of the experiments. For the work below room temperatures a cold stage was used (text fig. 1) which was constructed by the writer, by using 8-mm. glass tubing bent into a rectangular double coil allowing just sufficient space inside the coil for the easy placing and removal of a standard microscope-slide. A coil with two complete turns was found to have adequate cooling surface when properly insulated from without. The insulation was accomplished by gluing together several thicknesses of cardboard in the form of a rectangular box and cementing a glass window in the bottom over which the slide containing the hanging drop mount could be placed. The chamber was covered by means of two thicknesses of paper felt such as is used in drying plants for the herbarium. A hole was made in the center of these



TEXT FIG. 1. Low temperature chamber mounted on stage of a microscope ready for use, with intake at *A*, drain at *B*, thermometer at *C*, and weight *D*.

insulators just large enough to permit the insertion of the objective of the microscope and thus enable one to observe the mount without opening the constant temperature chamber. A thermometer was mounted with the bulb near the position of the slide in the chamber and with the part containing the scale protruding outside the insulating walls, thus enabling one to take temperature readings at will. The two ends of the coil were also allowed to protrude six inches or more outside the insulation. One end was connected to a cold water tap by means of rubber tubing, and the other allowed to drain into a sink. Text figure 1 shows this low temperature chamber mounted on a microscope stage with intake at *A*, drain at *B*, and thermometer at *C*. The weight *D* holds the chamber firmly on the stage of the microscope but permits movement of the apparatus; this enables one to orient the mount for observation under the microscope without affecting the temperature of the mount. With the temperature of the tap water varying around 13° C. it was possible easily to control the temperature of the chamber to as low as 17° C. by regulating the rate of flow of cold water through the coils. When temperatures slightly below 17° C. were desired, this could be accomplished by introducing a coil which was immersed in an ice bath between the water tap and the constant low temperature chamber. For the experiments in which it was necessary to have very low temperatures, as for example from 5° to 14° C., the most satisfactory results were obtained by working in a cold room with the temperature at 5° C. or lower. The temperatures above that of the cold room in which the experiment was set up were obtained by the use of the low temperature chamber; the water circulating through the coil in this case serves to raise the temperature to the desired degree. It was found most satisfactory to work in the cold room for all temperatures below 17° C. and raise the temperature of the low temperature chamber by circulating warm water through the coil.

For the study of the second swimming stage of the diplanetic species the most satisfactory and uniform results were obtained by removing the mycelium with the attached sporangia and replacing the water in which the spores of the first swimming stage had been liberated. This was accomplished by removing the cover glass and the hanging drop containing the material from the hollow ground slide. The mycelium and sporangia were then carefully removed after which the drop of water was drained away by means of a very fine capillary pipette. Since many of the spores come to rest on the surface of the drop one must exercise great care in order to avoid removal of the encysted spores. Well aerated fresh conductivity water was then added and the preparation returned to the slide and replaced under favorable conditions for further study.

The technique of killing and staining the zoöspores has been given by the writer in a previous paper (12). In the following work the technique used is essentially the same as that described for the zoöspores of *Blasto-*

cladia. In view of the fact that there has been some improvement in the staining technique since writing that paper, it may not be out of place to give a brief outline of the methods used in this work.

After the hanging drop containing the zoöspores had been exposed to the fumes of 1 percent osmic acid for from 30 to 60 seconds, an equal volume of a .005 percent aqueous crystal violet was added to the drop of spore suspension on a number one cover glass. The exposure to the osmic acid fumes and the concentration of the staining solution were varied occasionally in order to bring out the details of the structure of the different zoöspores. The preparation was then dried in a desiccator over sulfuric acid for from 24 to 36 hours. Spores which tend to take the stain less readily than others can be stained in a highly satisfactory manner by allowing the preparation to dry more slowly after the addition of the dilute staining solution. This allows more time for the stain to diffuse into the protoplasm of the spores. This was accomplished by simply allowing the preparation to air-dry either partially or completely. The final dehydration of the preparation was in all cases finished in the desiccator for all of the work carried on in the moist atmosphere at Ann Arbor, but in the dry high altitude of Bozeman, Montana, where part of the work was done, air-drying was sufficient.

In order to prevent the reabsorption of moisture, clove oil was added to the stained area on the cover glass as soon as the desiccator was opened and before removal of the preparation. After extraction and clearing in clove oil for from a few minutes to one half hour, depending on the rate of extraction, the oil was removed with xylol and the preparation mounted on a slide in balsam.

All detailed studies and drawings were made with the aid of a 2-mm. apochromatic oil immersion objective, a 12 × compensating ocular and a camera lucida.

EXPERIMENTAL AND CYTOLOGICAL STUDIES

Blastocladales

In this order the zoöspores are uniciliate and monoplanetic. Of the three species of this order studied *Blastocladia globosa* Kanouse and *B. pringsheimii* Reinsch have been reported by the writer elsewhere (12). *Allomyces arbuscula* is discussed in this paper. The genus *Allomyces* as well as the species were described by Butler (7) in 1911 and the following year collected and studied in some detail by Barrett (2).

Temperature Relations.—This plant is more sensitive to temperature than many of the other members of this group of highly sensitive plants. The temperature range of zoöspore formation and liberation extends from 18° C. to 32° C. Sporangia are formed slightly above and below this range but they seem to be unable to complete the development and liberate the spores at these temperatures. Sporangia which are formed just outside

the temperature range of zoöspore formation, *e.g.*, at 33° C., when later subjected to a temperature at or near the optimum will readily form spores in a perfectly normal manner. The optimum temperature for zoöspore formation in these plants lies between 25° and 27° C. At this temperature, sporangia begin to be walled off one hour after the washing process is completed, and the first liberation of spores takes place in from two and one half to three hours after completion of the washing process. It will be noted that the optimum temperature for formation of sporangia and zoöspores is somewhat nearer the maximum than it is to the minimum temperature. The optima for sporangium formation and for zoöspore formation seem to be the same, whereas the range for sporangium formation is somewhat greater than for zoöspore formation.

Optimum conditions and normal development are defined in this paper in the same way as in a previous paper dealing with the zoöspores of *Blastocladia* (12). *Normal development may be defined as the development which takes place under optimum conditions. Optimum conditions are those which enable the greatest possible number of active and healthy spores to be formed and liberated in the briefest given time.* The spores which develop under optimum conditions are believed to exhibit the morphology and behavior typical for the species.

Development of the Sporangia and the Structure of the Zoöspores.—The developing sporangium is first observed as a slight swelling of the tip of the hypha; a slight constriction may be seen to develop a short distance from the tip indicating the position of the developing cross wall which forms as a ring from the periphery of the hypha toward the center. The walling off of the first sporangia under optimum conditions begins about one hour after completion of the washing process. The entire process of septum formation requires from one-half to three-fourths of an hour for completion. The maturation of the young sporangia takes place rapidly and under favorable conditions spores will begin to be liberated in from one and one-half to two hours after the first indication of developing cross walls. As described by Barrett (2), liberation of the first spores takes place into a vesicle, which persists until from four to six spores have emerged from the sporangium; the vesicle then ruptures, liberating the spores.

The zoöspores of *Allomyces arbuscula* have been described in some detail by Butler (7) and by Barrett (2). There are, however, a number of cytological details which need further consideration. Several striking similarities have been noted between the zoöspores of this plant and the uniciliate spores of the two species of *Blastocladia* studied by the writer (12). But there are also a number of significant differences; this necessitates the complete redescription of the zoöspores of this species.

The first spores that emerge from the sporangium are at first irregular in shape and often exhibit amoeboid changes as described by Butler. The immature cilium, if any has developed at this stage, is much shorter and

thicker than the cilium of the mature active spore. Plate XXX, figure 1, shows a spore with an immature cilium. It is quite evident that the development of the cilium is in some way intimately connected with one or several highly staining granules. There is always a main granule which occupies the position at the base of the cilium, and there are in addition often as many as three secondary granules which occupy positions at the base of threads connecting the basal granule and the nucleus (figs. 1 and 2). Many young spores show but one secondary granule and one thread connecting it with the basal granule. After the completion of the cilium, which seems to be accomplished by its being whipped about until it has attained sufficient length and delicacy to function as a propeller, these smaller granules, sometimes three in number, are drawn back toward the nucleus. In combination with several other chromatic granules they seem to form the tip of this organ (fig. 3).

There are also very definite connections between these secondary granules and the large central chromatic body in the nucleus. This body in turn is connected to the peripheral chromatic granules of the nucleus by delicate threads, so that the result is a rather coarse and meshed net-like structure (fig. 1). The peripheral chromatin is distributed in various-sized granules just inside the nuclear membrane. The nucleus is very similar in shape to the nuclei of the zoöspores of the species of *Blastocladia* studied; it is, however, usually somewhat smaller in the spores of *Allomyces arbuscula*. The rather large, very finely granular, cytoplasmic mass, lying just forward of the broader anterior part of the nucleus in which this broader part of the nucleus is imbedded, is considered by the writer to be homologous with the finely granular, dense mass of cytoplasm which has been described (12) for the spores of the genus *Blastocladia*. In favorable material of *Blastocladia* this forms a cap-like structure between the anterior end of the nucleus and the surface of the spore. This cap has been referred to as the food body by Barrett (l. c.) and is shown by him as a definitely outlined body limited by a membrane. Under certain conditions and particularly in the older spores this finely granular cytoplasmic body does appear to have a very definite exterior boundary line, while in younger material this boundary is often not well marked (figs. 1 and 3). This question will be discussed in more detail a little later in the paper. In the young spores the highly vacuolated cytoplasm of the extreme anterior end is often drawn out into a rather long-pointed tip. This condition may possibly be brought about in some cases by the young spore being forced out, ciliated end first, through the exit-pore of the sporangium, resulting in the crowding of this more pliable material toward the anterior end of the spore. In other cases this end may have been drawn out by becoming attached to the cilium of the spore immediately ahead of it. The latter explanation is probably more generally true, especially for those spores emerging later and after the development of the cilia. But whatever the

orientation of the young spores, this extreme anterior end is filled with highly vacuolated cytoplasm which contains many large granules that are either distributed through it (fig. 2) or are congregated about the denser cytoplasm (fig. 4). At favorable temperatures, after the spores have been allowed to swim for several hours, most of this highly vacuolated cytoplasm along with the large granules has moved back around the equatorial region of the spore; or it may even pass beyond this region toward the insertion of the cilium. In the young spores the equatorial region is not filled with these large granules. This change in position is probably brought about by the motion of the spore through the water, which crowds back the less dense cytoplasm, containing the large granules, from the forward end of the spore. Such a movement of the peripheral cytoplasm back, over, and around the denser cytoplasm results in a more clear-cut boundary of the dense cytoplasm in front of the nucleus. Figures 5 and 6 show spores which had been allowed to swim a number of hours at the optimum temperature. It will be noted that most of the cytoplasm containing the large granules has been crowded back or migrated away from the extreme anterior end of the zoospore. The size of the zoöspores of the plants studied at Ann Arbor corresponds very closely to the measurements given by Barrett.

Saprolegniales

In this order the zoöspores are biciliate and either monoplanetic or diplanetic. From the point of view of zoöspore activity, three distinct groups of plants are included here: (a) the plants in which the zoöspores are active in both the first and second swimming stages, represented in this study by *Saprolegnia monoica* var. *glomerata*; (b) the plants in which the zoöspores may or may not be active during the first swimming stage but are always active after encystment. These fungi are represented here by *Isoachlya paradoxa*; (c) the plants in which the zoöspores are never active during the first swimming stage. The types used here were *Achlya conspicua* and *Aphanomyces euteiches*.

Saprolegnia monoica var. *glomerata*

The genus *Saprolegnia* was described by Nees von Esenbeck (33) in 1823. The species under discussion was described in 1858 by Pringsheim (36) and the variety in 1912 by Tiesenhausen (43).

Plants belonging to the genus *Saprolegnia* are very favorable material with which to work since they are easily collected, isolated, and carried in pure culture. They were therefore utilized for the perfection of much of the technique used in both the physiological and the cytological studies of the zoöspores.

Temperature and Oxygen Relations.—The studies of zoöspores of the species of the genus *Saprolegnia* have evidently been made by most students at whatever temperature happened to prevail in the room or culture at

the time that the observations were being made. No mention is made in the literature of an attempt to control accurately the temperature during zoöspore formation in these plants, and scarcely any clear-cut temperature data are available.

It was observed at various times by the writer that there is a relation existing between zoöspore activity and air, since zoöspores liberated or mounted in a hanging drop are more active and collect in greatest numbers near the surface of the drop where it is assumed they are able to absorb oxygen more readily. A number of experiments were therefore set up, in order to determine the effect, if any, that might be produced by the thorough aëration of the conductivity water prior to washing and mounting the young vigorous mycelium in it.

When two lots of mycelium, similar in all respects, except that one lot was washed and mounted in sterilized aërated water whereas the other was washed and mounted in sterilized but non-aërated water, it was found that the aërated culture formed and liberated zoöspores in one half to less than one half the time that was required by the non-aërated. Thus, at or near the optimum temperature for *Saprolegnia monoica* var. *glomerata*, spores would be liberated in well-aërated water after one and one-half hours. In the case of the non-aërated material three hours elapsed before any spores were liberated. Under less favorable temperatures but within the range of zoöspore formation, we get similar results, but the process takes a longer time in both aërated and non-aërated material. For example, at 26° C., one and one-half hours was the time required for aërated material and three hours for non-aërated material; at 22° C., three hours for aërated and six and one-fourth hours for non-aërated material; at 18° C., four and one-fourth hours for aërated and seven and one-half hours for non-aërated material. The results show that there is a very definite and consistent speeding up of the process of sporangial formation and zoöspore liberation when the material is well aërated.

The temperature range for zoöspore formation in *Saprolegnia monoica* var. *glomerata* ranges from below 16° C. to 32° C. The optimum temperature for zoöspore formation among plants belonging to this species lies very close to 26° C. At the optimum temperature sporangia are walled off one half hour after washing is complete and spores are beginning to be liberated one and one-half hours after completion of the washing process. The time required for the formation and maturation of the sporangia increased as the temperature at which the experiments were run was removed from the optimum. The most rapid increase of the time required for zoöspore formation is toward the maximum temperature.

Structure of the Zoöspores.—The gross morphology and ciliation of the zoöspores of species of *Saprolegnia* have been described repeatedly in the literature. The cytological details of the zoöspores however seem to have been almost completely neglected. De Bary (5) speaking of the cilia says,

"flagellum or one or two slender cilia with the power of lively motion spring from a definite spot in their surface as processes of the peripheral layer of protoplasm." Rothert (39) has shown that the cilia appear at first as slow outgrowths like little short straight bristles and that these show faint oscillations. No details of the relation to the organs of the cell, of either the young developing cilia or the mature active cilia, have been shown for members of this genus.

Each of the two cilia of the spores of the first swimming stage of this species has its insertion in a highly staining granule just beneath the plasma membrane at the smaller end of the spore. These basal granules seem to terminate the beak-like extension or extensions of the nucleus (fig. 14). That the main body of the nucleus is also well differentiated is shown in figure 14. A clearly defined dense chromatic body may be observed near the center of the nucleus in some of the spores. The central dense body is connected with the peripheral chromatin, which is situated just inside the nuclear membrane, by several very fine almost hyaline strands. Two of these strands uniting the central and peripheral chromatin seem to connect, one with either side of the base of the beak-like structure. The tip of this structure is terminated by the basal granules at the insertion of the cilia.

It will be noted that the nucleus is much nearer to the surface of the spore as represented in figure 15 than in the one shown in figure 14. The position of the nucleus as shown in figure 15 is the more typical one for the young spores. In the case of the older spores the nucleus has often moved back some distance toward the center of the cell. This condition is especially true of the zoöspore which is about to go into the encysted period following the first swimming stage. Spores were occasionally found in which the basal granules at the insertion of the cilia were somewhat wider apart than usual (fig. 16). It is evident from the spore represented in this figure that there are separate connections between the nucleus and each of the two basal granules. It is also evident that these connecting threads have their insertion on the main body of the nucleus in separate places which in this case are in definite chromatic granules which in turn are connected with a common chromatic mass. The chromatin in which each of the cilia have their final origin is in this case somewhat V-shaped with the tips of the V connected with the basal granules by definite strands.

The cytoplasm of these spores is divided into two rather distinctly differentiated parts. The central and more dense part surrounds the main body of the nucleus and extends through the central part of the body of the spore. There are numerous highly staining granules throughout this rather well differentiated central mass of cytoplasm. The peripheral zone of cytoplasm surrounds this central, more dense portion, and contains many small vacuoles. The peripheral cytoplasm is considerably more extensive in the anterior part of the spore body, causing an enlargement of this part of the spore; this results in the typical pear-shaped structure of these zoöspores.

Giant spores may be found occasionally (see general discussion for the physiological explanation of this phenomenon). Figure 17 shows one of these giant spores. Two nuclei are present, each, with its two cilia, having the typical organization of the species. These spores are very evidently the result of incomplete cleavage of the protoplasm in the sporangium.

As the spores emerge from their cysts, in preparation for the second swimming stage, the nuclear apparatus is observed to be considerably elongated and curved so as to appear somewhat crescent shaped. A granule may be seen at either tip. The tips of this curved nucleus approach or touch the plasma membrane. At a slightly later stage two finger-shaped pseudopod-like protuberances may be observed to have developed at points about as far apart as the distance between the tips of the curved nuclear apparatus and evidently at the places where the tips of the nucleus approached the plasma membrane (fig. 18). A somewhat later stage is shown in Plate XXXI, figure 19. The pseudopod-like projections have now become united and the one end of the nucleus has retreated somewhat from the granule which formerly was connected with it; this part or extension of the nucleus is beginning to round off and to be drawn back into the main body of that organ. In figure 20 we find the young cilia in the form of a loop, one side of which is much heavier than the other. There is also a small mass of colorless material which seems to have collected at the outer extremity of the loop. In figures 21 and 22 the cilia are observed to be almost mature. It will be noted that one is heavier, and in one case longer, than the other and that one is also more intimately connected with the nucleus. This may probably be explained by the unequal behavior of the nuclear apparatus in the very early stages of the development. The cilium which is most intimately connected with the nucleus probably developed in proximity to the basal granule, which did not become separated from the nucleus early in the cilia formation process, as did the basal granule of the other cilium.

The nucleus of the spore of the second swimming stage now becomes somewhat more definitely organized (figs. 23 and 24). The peripheral chromatin is often more definitely arranged toward the larger or anterior end of the nucleus (fig. 23). The cytoplasm of this, the second swimming stage, is divided into two distinct parts, an inner more dense portion and a peripheral less dense portion. The inner, more dense cytoplasm surrounds the nucleus and has irregularly pointed projections extending outward into the peripheral or less dense zone of cytoplasm. There are occasional vacuoles and some deeply staining granules found in this central cytoplasm. The peripheral cytoplasm is much less dense and contains many irregularly arranged vacuoles. The outer boundary of the peripheral cytoplasm in the younger spores is very irregular but becomes more regular as the spore matures. Occasionally, in preparations of these spores, deeply stained granules are found scattered through the peripheral cytoplasm.

Isoachlya paradoxa

This species was first described as *Achlya paradoxa* by Coker in 1914. Speaking of this plant he says, "so puzzling is the form that after preparing a description of it in 1912 it was decided to continue collections and experiments for another year before publication," and further, that "The difficulty arises from the fact that our plant combines in a most confusing manner the characters of both *Achlya* and *Saprolegnia*, and a rigid interpretation of these genera as at present defined would exclude it from both"; he concludes the paragraph by saying that "as the proliferation is usually of the *Achlya* type I have decided to refer this form to the genus *Achlya*." Kauffman (26) in 1921 described the genus *Isoachlya* and included this species along with others, all of which, he states, naturally fall within the boundaries of this genus. In 1923 Coker (9) erected the new genus *Protoachlya* for this single species. In speaking of this new genus he says, "It seems almost exactly intermediate between *Saprolegnia* and *Achlya*," and "The genus normally exhibits not only characters of several genera, but combines what has been considered antithetic characters, as both cymose and inter-sporangial proliferation of sporangia and motile and motionless spores on emerging." It is apparent from the description of the genus *Isoachlya* that it was formed to include plants of just such characteristics as those just quoted. From the studies described below, it becomes evident that the genus *Isoachlya* is broad enough to include all of the interesting variations of this species also.

Temperature Relations.—The optimum temperature for zoospore formation and liberation for this plant lies between 23° and 25° C. The maximum temperature for this process lies between 32° and 34° C. Within the optimum range of temperature zoospores are formed and liberated in from 4 to 6 hours. At 29° to 31° C., it requires from 10 to 12 hours for the completion of this process; a similar time is required at 16° to 18° C. Further lower limits for zoospore formation for this species were not studied.

Under conditions near the optimum temperature for zoospore formation it is found that there is but slight tendency toward the *Achlya* characteristics of this species. The spores under these conditions are expelled from the sporangium in a manner almost typically that of *Saprolegnia*. They do however, tend to hesitate, as it were, a short distance from the mouth of the sporangium seeming to wait for the entire group to become liberated from the sporangium. During this interval, of but a few seconds duration, the spores are in a state of constant agitation. They then swim away in the typical manner known for *Saprolegnia*. The secondary sporangia under these conditions are invariably formed by internal proliferation as in *Saprolegnia*. Spore formation in *Isoachlya paradoxa* may be quite unique. Where this occurs in a hanging drop preparation and when the conditions are as nearly optimum as possible, one can find in certain occasional

sporangia, spores which after liberation slowly creep and rock back along the wall of the sporangium and finally encyst there. Such a behavior is only observed in sporangia situated well on the interior of the small mass of mycelium of the mount, and occurs after other sporangia have been forming and liberating spores for a time. It would seem that this is probably the result of reduced aëration in this region of the mount.

By proper handling of the material dictyosporangia may be obtained in this species. These are produced experimentally by allowing the sporangia to develop just within the upper conditioned limit of zoöspore formation. They are then held for a few hours at a temperature just above this limit and finally brought to their optimum temperature. The formation of dictyosporangia under these conditions is explained by the fact that the spores which have been formed in the sporangia encyst there when placed under temperature conditions unfavorable for their being liberated. Then after again being placed under favorable conditions for spore liberation they are freed from their cysts through the old sporangial wall. When the spores have been prevented from escaping in a normal manner by being placed under somewhat unfavorable conditions the sporangiophores tend to form secondary sporangia as in the genus *Achlya*. The *Achlya* type of sporangial proliferation seems to be more prevalent on plants grown on flies than when developed from a young vigorous mycelium grown on dilute pea broth. Aside from the difference of constituents between the two food materials, in the case of the pea broth culture, food may be absorbed by any part of the entire mycelium, while in the case of the plants growing on the fly the major part of the nutrition must come from the attachment of the mycelium in the body of the fly. At any rate there is a marked difference noted between plants grown on the two food materials which emphasizes the importance of a standard substratum for this group of plants in order to be able to understand and interpret the various results reported by investigators.

Zoöspore formation and liberation as well as sporangial proliferation take place, under optimum conditions, in a manner almost typically that of *Saprolegnia*. If, however, plants are kept under conditions approaching the limits of zoöspore production, it is found that they tend to take on more of the characteristics of the genus *Achlya*. It seems that when the sporangia are produced under conditions which do not favor the prompt liberation of their zoöspores, the protoplasm of the hypha supporting the sporangium becomes active before the sporangium is emptied, resulting in the initiation of a lateral proliferation. It is apparent therefore, when studying the species of this genus which are even more sensitive to environmental changes than other members of the Oömycetes, that it is necessary to indicate clearly all of the conditions to which the plants are subjected. If this is done, then a genus such as *Isoachlya*, which also has characters of other genera, depending on the conditions under which the plants are allowed to develop, takes its proper place in plant classification.

Structure of the Zoöspores.—The cytological details of the zoöspores of these plants are similar in all respects to those described in detail for *Saprolegnia*. It seems therefore a waste of time and space to enter into a detailed discussion of the structure of the spores of this species.

Achlya conspicua

The genus *Achlya* was described by Nees von Esenbeck (33) in 1823. The species under discussion was described by Coker (9) in 1923.

Temperature Relations.—The optimum temperature for the formation and liberation of the zoöspores lies between 23° and 27° C., with slightly better indications between 25° and 26° C. At these temperatures sporangia are formed and spores liberated in from 4 to 5 hours. Sporangia are walled off in slightly less than 8 hours at 34° C. but no spores were observed to be liberated at this temperature. Spores are liberated in 6½ hours at 18° C. and in from 22 to 24 hours at between 10° and 12° C. The reactions at lower temperatures were not studied.

Structure of the Zoöspores.—In the developing sporangium at a stage during or somewhat later than the "homogeneous" stage of Büsgen (6), or the "stage of swelling of the spores" of Rothert (39), the nuclei may be observed to be in a state of activity. Humphrey (22) speaking of this stage says, "In spots corresponding approximately to the middle of the spore origins are to be seen clear, bright spots, and throughout the whole protoplasm are numerous vacuoles which appear and disappear shifting about rapidly." If a mass of these spore initials are killed and stained at this stage of development the nuclear apparatus may be observed to be in the form of a crescent-shaped spindle (fig. 25). The bulk of the chromatic material will be observed to occupy a position midway between the poles. At each pole, in favorably situated nuclei, may be seen one or more granules (fig. 25 *a*). In some of the spore initials these granules at the poles of the spindle have been brought into close proximity with the surface of the spore initial (fig. 25 *b* and *c*). These granules are the basal granules from the region of which the cilia develop. The poles of the nuclei and the accompanying granules are often at almost opposite sides of the spore initials (fig. 25 *c*). These granules apparently must migrate around toward each other until in the first swimming stage they occupy positions close together. Part of this migration evidently takes place after the cilia have started to develop. Couch (13), in agreement with Cornu (11) and Rothert (39), has shown that the spores of several species of this genus have connecting threads between the emerging spores. In the species studied by the writer such definite connections were found, at the same stage (fig. 26). Couch (13) in his discussion of these connecting threads states that "on other spores these threads were seen moving around from the opposite pointed ends of the spores approaching each other and on still others these threads had come to occupy the position of cilia."

The writer believes it to be a matter of chance whether the cilia happen to be connected with a thread in the process of their development. When spores are allowed to be formed under optimum conditions the cilia are quite immature at the time the spores are liberated from the sporangium (figs. 27 and 28). If we examine figure 28 it will be seen that the cilium (*a*) has developed relatively near to the attachment of the connecting thread (*b*). If we imagine this cilium developing directly underneath this connecting thread then in all probability the connecting thread might have developed into a cilium. The cilia which are at least partially developed at the time that the spores are liberated do not seem ever to develop sufficiently in this stage in this genus to function as organs of locomotion. They are soon absorbed as the spore prepares to enter the encysted stage. Figure 29 shows three spores which are preparing to enter the encysted stage. In the spore (*b*) it will be noted that the basal granules have been pulled toward the nucleus resulting in a drawing in of the plasma membrane. In the spore (*c*) the basal granules have broken away from the plasma membrane and have migrated some distance toward the nucleus. Figure 30 shows a spore in which the nucleus has almost reached a spherical shape as the spore enters the encysted stage.

The zoöspores of the second swimming stage of this species seem to be quite similar to those of *Saprolegnia* and *Aphanomyces*. The cilia are developed in the same manner as described and figured in some detail for these two genera. Figure 31 shows a spore in which the young cilia are almost free from the bit of debris to which the extreme ends still cling. Note the waves which evidently move out along the cilia from their base toward the tip. In the mature active spore the cilia may be observed to be inserted in basal granules which are connected with the nucleus (fig. 32). The cytoplasm surrounding the nucleus is more dense and less vacuolate than that nearer the periphery of the spore. Numerous larger granules may be observed in various parts of the cytoplasm.

Aphanomyces euteiches

The genus *Aphanomyces* was described by de Bary (3) in 1860. The species under discussion was described by Drechsler in Jones and Drechsler (24) in 1925 and has been shown by them to be the pathogen causing a serious root rot of peas.

Temperature Relations.—Jones and Drechsler report that the temperature range for spore liberation from the sporangial filaments extends from between 8° and 9.5° C. to between 33° and 35° C. and that the range of zoöspore activity extends from between 9° to 11° C. to between 21° to 22° C. At the lowest temperatures the zoöspores did not appear until after 49 hours or more time had elapsed. The optimum temperature for zoöspore formation and activity in this species was found by the present writer to lie between 26° and 28° C. As a matter of fact the range of

optimum temperatures for this process here really extends from 25° to 29° C. with slightly better indication between 26° and 28° C. The maximum temperature for spore formation was found to lie between 31.5° and 33° C. This it will be noted is a slightly lower maximum than was found by Jones and Drechsler. However the difference is readily explained by the fact that the writer did not extend his experiments on this point beyond 24 hours, since his chief interest was to find the optimum conditions for the production of normal typical zoöspores of the species. Some of the time relations to zoöspore formation at different temperatures as found by the writer for this species are as follows: at 17° to 18° C. more than 12 hours were required; at 19° to 21° C., 6 to 7 hours; at 21° to 23° C., 4½ to 5 hours; at 24° to 25° C., 4¼ hours; at 25° to 27° C., 4 hours; at 26° to 28° C., 3¾ hours; at 27° to 29° C., 4 hours; at 28° to 29° C., 4 hours; at 31° to 32° C., 7 hours; at 31.5° to 33° C., no zoöspores were formed.

The importance of favorable temperatures for the natural rapid development of *Aphanomyces euteiches*, in the presence of favorable moisture conditions, has been pointed out by Jones and Drechsler (24). In their investigation it was shown that an unusually rapid development of the root-rot caused by this fungus appeared following a period of five days during which time the mean daily soil temperature rose from 17.5° to 20.5° C. with the minimum for the period above 15° C. It will be noted that the soil temperature here reported approached to within less than six degrees of the optimum conditions for zoöspore formation and activity. At these soil temperatures zoöspores may be formed in from 6 to 7 hours. Since these soil temperatures are the mean average temperatures, in all probability the temperature during the warmer part of the day was very close to the actual optimum temperature. Since it requires less than four hours for the formation of spores at this temperature and since the soil temperature for the entire period was within the range of zoöspore activity, and provided there was a considerable quantity of mycelium present, the enormous possibilities of infection become apparent.

Structure of the Zoöspores.—The morphology of the first swimming stage of the forms of this genus and the discharge of the young spores from the sporangial filaments has been well described at different times by several botanists, viz. de Bary (5), Rothert (40), Couch (13), Jones and Drechsler (24). The writer is in agreement with these workers as to the fact that no cilia are found on the spores of the first swimming stage of this plant. Plate XXXII, figure 33, shows a spore which has recently emerged from the sporangial hypha and has rounded off in preparation for encystment. The thread-like appendages which formerly connected this spore with its neighbors may still be observed. It may also be noted that the nucleus occupies a central position at this stage of development.

This genus is particularly favorable for detailed studies of the development of the spores as they enter the second swimming stage. Since, under

favorable conditions, large numbers of spores are liberated through a single exit pore, and since they do not have any means of locomotion, they remain in close proximity to each other until after they emerge from their individual cysts. It is apparent then that we may have large numbers of spores emerging and going into the second swimming stage in a small area of the mount and over a short period of time. This enables one to have a wealth of material for study, very favorably located. The spores were allowed to develop in hanging drops which may be continuously observed, and killed and stained as desired.

Following the encystment of the zoöspores after their liberation from the sporangial filaments, the spore passes out of its cyst, the gross features of which have been well described by Jones and Drechsler (24). During or immediately following the process of emergence from the cyst the nucleus of the spore is observed to be the center of activity. The nucleus forms a spindle-like structure with several larger chromatic granules near the center and from one to three smaller granules near each of the poles. The entire structure is more or less curved, either crescent-shaped (fig. 34 *a* and *b*) or with two leg-like projections (fig. 34 *c*). In some cases a small pseudopod-like structure may be observed to project out from the surface of the spore opposite one or both poles of the nuclear apparatus (fig. 34 *a* and *b*). In figure 34 *a* all of the granules have been separated from one end of the spindle-like structure. This separation does not always occur, in fact it does not seem to be a usual occurrence at all as will be shown in the discussion of some of the later stages of these spores. The pseudopod-like extensions elongate very rapidly and by being lashed about often come into contact with some object to which they then adhere (figs. 35 and 36). The entire surface of the spore is quite irregular at this stage, numerous short protuberances being evident. This would seem to indicate that the entire peripheral part of the spore is in an active state. During the maturation of the cilia they seem to be in the condition of being stretched out and the granular elements, which had migrated out into them in the pseudopod-like stage, now migrate back toward the spore body and the cilia are soon torn loose from the foreign material with which they are in contact and the spore swims away. Figure 37 represents a spore which seems to have torn itself almost free from encysted spores with which its young cilia had come in contact. In some cases when the young cilia do not come in contact with another object they may stick together at their tips and by being lashed about they are stretched out in this loop-like manner (figs. 38 and 39) and are evidently finally torn apart so as to result in the usual two separate cilia. During the early stages of the maturation of the cilia the nuclear connections are not as evident as they are later, and the nuclear activity or migration of the granules seems to be away from the body of the nucleus. In the later stages, when the granular material seems to be moving back toward the body of the spore, the connections to the main

body of the nucleus become more evident. Occasionally the nuclear connections are much more pronounced toward the base of one cilium. An explanation of this is suggested by figure 34 *a* where the granules at one pole of the nuclear apparatus were separated at this early stage. Figure 40 shows a more mature spore in which one cilium seems to be entirely separate from the main body of the nucleus. The cilium is evidently controlled by the chromatic granules connected with the basal granule. These granules, it is assumed, were thrown off from the nucleus in a manner similar to that shown in figure 34 *a*. In many of the mature spores both cilia are found to be in direct connection with two parts of the nucleus (fig. 41). This is interpreted as a later development of the condition shown at an earlier stage in figure 34 *b* and *c* where both of the basal granules remained in contact with the tips of the nucleus at least until later in the development of the pseudopod-like projections of the cytoplasm. In many of the spores the opposite poles of the nucleus, where the granules were not dropped by one pole, have evidently moved around and united to form a single beak-like extension (fig. 39). In this case the basal granules often seem to have fused to form an elongated and curved chromatic rod connecting the two cilia at their bases and connected to the beak-like extension of the nucleus (fig. 39). There is a denser chromatic body which is situated in the beak-like extension and is connected with the basal granule by a definite strand. When there are two beak-like extensions of the nucleus as in figure 41 each has a denser chromatic granule; one, however, is likely to be much more massive than the other (fig. 41). The nucleus is shaped almost like an Indian club with the smaller end representing the beak (figs. 39 and 40) or when two beak-like structures are present it usually has the general shape as shown in figure 41. The cytoplasm of these spores is denser around the nucleus and gradually becomes less dense and more vacuolate toward the periphery of the body of the spore. There is a less clear-cut differentiation between the central and the peripheral cytoplasm of the spores of this plant than is found in the spores of *Saprolegnia monoica* var. *glomerata*.

Leptomitales

In this order the zoöspores are biciliate and either monoplanetic or diplanetic. From the point of view of zoöspore activity, this order includes two distinct groups of plants, (*a*) the diplanetic genera represented in this paper by *Apodachlya brachynema* and (*b*) the monoplanetic genera represented by *Rhipidium europaeum*.

Apodachlya brachynema

This plant was described in 1867 by Hildebrand (20) as *Leptomitius brachynema*. In 1883 it was placed in the genus *Apodachlya* by Pringsheim (37) when he described the genus.

Temperature and Oxygen Relations.—The optimum temperature for zoöspore formation lies between 15° and 19° C. Within this range sporangia are formed and zoöspores liberated in from 9 to 10 hours. Sporangia are formed in great abundance up to temperatures between 26° and 27° C. but zoöspores are rarely liberated above 22° C. Sporangia are formed at temperatures as low as 10° C. and spores liberated in considerable numbers at this temperature after 28 to 30 hours. In this species there is an unusually wide range within which sporangial formation takes place above the temperature limit for zoöspore liberation.

Considerable difficulty was experienced at first in obtaining spores of the second swimming stage. It was found, however, that after spores of the first swimming stage have come to rest and are completely encysted the second swimming stage can be initiated by replacing the water of the hanging drop of the mount with water that has been thoroughly aerated. The water which is used to replace the vitiated water of the mount should be aerated at or below the temperature of the mount. Within the optimum range of temperature encysted spores treated with newly aerated water will begin to emerge vigorously from their cysts at the end of one hour.

Structure of the Zoöspores.—The zoöspores of the first swimming stage of this species exhibit the most uniform and characteristic ovoid shape of any of the biciliate spores studied. These spores are nearly symmetrical, usually, however, with a slightly flattened area on one side near the insertion of the cilia, giving to the spores a slightly lop-sided appearance. The cilia are two in number, comparatively short, each having its insertion in a separate basal granule. The basal granules are connected with the tip of the nucleus by definite thread-like structures. The nucleus is oval in shape with an almost beak-like tip in which may be found the denser chromatic body which is connected with the basal granules. The cytoplasm is very finely granular and contains numerous small vacuoles; the latter are more prevalent in the peripheral and anterior part of the cell (Pl. XXX, fig. 7).

The spores of the second swimming stage are ovoid in shape. The two cilia are inserted laterally in definite basal granules; the latter are either separate (fig. 7) or appear to be fused (fig. 8). Definite connections may be observed between the basal granules and the nucleus. The nucleus also is ovoid in shape and irregular chromatin granules are distributed in its peripheral region. Occasional spores may be observed with the nuclei slightly curved (fig. 10). Near the tip of the nucleus larger and denser granules are usually evident at the insertion of the strands connecting this organ with the basal granules. The cytoplasm is very finely granular, with occasional very small vacuoles. In some spores the peripheral region of the cytoplasm is found to contain many granules slightly coarser than those of the central cytoplasm (fig. 8).

Rhipidium europaeum

The genus *Rhipidium* was described by Cornu (10) in 1871. His original species were *R. continuum* and *R. interruptum*, which were connected by von Minden (32) and probably conceived by him to be equivalent to *R. europaeum*.

Temperature Relations.—No reference is made in the literature to any observed temperature relation to the formation and liberation of zoöspores in the species of this genus. Scarcely any illustrations of the liberated zoöspores of these plants are found among the various illustrations of plants belonging to this genus. One naturally wonders regarding this dearth of illustrations of an important stage in the life history of an entire genus of plants. From what follows below, it is easy to infer that such descriptions and illustrations of these plants as are found in the literature have probably been made from plants observed under conditions for zoöspore formation other than the optimum (Minden, 32, and Myk. Untersuch. und Ber. 2: 146-254. 1916; and Thaxter, Bot. Gaz. 21: 317-331. 1896).

The temperature range for the formation of zoöspores and their liberation in this plant is from about 12° C. to slightly above 22° C., a total range of but ten degrees centigrade. The optimum temperature for zoöspore formation and activity lies between 16° and 18° C. The fairly low temperature range of zoöspore formation and liberation is probably the explanation for the dearth of illustrations. If plants are kept at near the maximum temperature for this process, under rather unfavorable conditions of aëration, zoöspores are formed but not liberated. This emphasizes the importance of complete control of environmental conditions when studying the life history of these highly sensitive plants.

Structure of the Zoöspores.—The motile spores of this monoplanetic plant exhibit the same general form and structure as those found in the second swimming stage of related genera. There are, however, some characteristic details, serving to distinguish the spores of this plant, which will be discussed as we study the details of the different parts of the motile spores. The cilia have their origin in a single granule or a complex of basal granules (fig. 11). The granules are in either case united with a more massive chromatic structure situated adjacent to the nucleus. The nucleus in the more mature spore, when viewed from the side, has much the same organization as that described elsewhere in this paper for the spores of the second swimming stage of *Saprolegnia monoica* var. *glomerata*. The cilia may continue to be connected with the almost opposite ends of the nucleus (fig. 12) or the cilia, along with the basal apparatus, may have migrated around to a lateral position near to each other (fig. 13). The peripheral chromatin in the nucleus of a spore, with its parts oriented as in figure 13, when viewed in optical section, appears to be shaped almost like a horseshoe, with the toe of the shoe occupying the position of the broader or anterior part of the nucleus. The mass of chromatic material

near the tip of the nucleus is somewhat curved, and the ends of the curve point toward the basal granules at the insertion of each of the two cilia. This chromatic material is evidently a product of the fusion of two masses similar to those shown at the base of each ciliary apparatus in figure 11.

The central cytoplasm surrounding the nucleus is less extensive than that found in the species of *Saprolegnia* studied. The peripheral cytoplasm on the other hand is more extensive than was found in other zoöspores. There are many very regular and clear-cut vacuoles distributed throughout this region of the cell. They are usually arranged in a single layer just inside the plasma membrane. The framework of cytoplasm filling the spaces between these vacuoles seems to be of a denser nature than that found in the peripheral region of the other biciliate spores. Cytoplasm filled in this fashion with a rather regular array of vacuoles gives to these spores a coarse, rough appearance, even when observed in the active living condition.

Peronosporales

In this order the zoöspores are biciliate and monoplanetic. The order is represented in this study by five species of the genus *Phytophthora*; one species, *P. palmivora*, is studied cytologically.

Phytophthora

The genus *Phytophthora* has attracted the interest of mycologists and pathologists since its description by de Bary (4) in 1876. This interest is probably due first to the economic significance of the various species of the genus, and second because the critical separation of the species of this genus is not promoted by the presence of sharp morphological characters.

Temperature and Aëration in Relation to Zoöspore Formation.—Among the studies reported in the literature regarding the effect of various degrees of temperature on the formation and activity of the zoöspores of this genus are those of Dastur (14) who has shown that in *P. parasitica* Dastur the zoöspores are liberated from sporangia in five minutes at 25° C. and that higher temperatures retard the formation of zoöspores. Melhus (31) who studied zoöspore formation in *P. infestans* (Mont.) de Bary, has shown that the optimum temperature for zoöspore formation in this species lies between 12° and 13° C., with the minimum between 2° and 3° C., and the maximum between 24° and 25° C. Gadd (16), in his work with *P. faberi* Mont., has shown that the optimum temperature for zoöspore formation in this species lies between 20° and 25° C. and that zoöspore formation is retarded below or above these temperatures. He has also shown that well-aërated water is necessary for the formation of zoöspores in large numbers. Ashby (1) working with *P. palmivora* says, "When the mature sporangia from cultures five to seven days old were brought into cool well-aërated water, a number showed segmentation of the granular contents and discharged zoöspores within fifteen to thirty minutes."

The complete temperature range for zoöspore formation was obtained by the writer in the study of an undescribed species of *Phytophthora* obtained from Dr. D. Reddick. This species, along with *P. palmivora*, *P. terrestris*, and others, has been combined into de Bary's *P. omnivora*, in the studies of this genus reported by Leonian (29). This procedure seems to be questionable in the light of our far from complete knowledge of the group. The temperature range of zoöspore formation in this species extends from between 4° and 6° C. to between 34° and 36° C. with the optimum between 25° and 27° C. At the optimum temperature spores are liberated in well-aërated water in five minutes. At temperatures removed from the optimum the time required for zoöspore liberation increases to an hour or more near the limits of zoöspore formation for the plant.

In *P. erythrosepica* the relation of zoöspore formation to aërated and non-aërated water was also studied. The temperature relation was found to be very similar to that just shown for Reddick's *Phytophthora*. It was found that at the optimum temperature spores were liberated in five minutes when using well-aërated water and were not liberated until after more than three hours when using non-aërated water.

The plants of *P. cactorum* and *P. terrestris* showed reactions similar to those of the species just discussed, excepting that the minimum time for zoöspore formation for these two species was found to be ten minutes instead of five minutes.

The writer has found that the optimum temperature for zoöspore formation in *P. palmivora* lies between 23.5° and 25.5° C., at which temperatures zoöspores were liberated in nine to ten minutes. As the temperature at which the preparation is kept is gradually moved from the optimum, there is an increase in the time required for the formation of zoöspores. Thus between 25.5° and 27° C., it requires seventeen to eighteen minutes for the liberation of spores; between 27° and 29° C., it requires twenty minutes; between 29° and 31° C., it requires from thirty to sixty minutes. Above 31° C. zoöspores were not formed.

In view of the fact that the temperature range for zoöspore formation is distinctly different in this species and that its optimum falls below the optimum for Reddick's *Phytophthora* and for *P. terrestris*, and since these species also have morphological and pathogenic differences which cannot be disregarded, it becomes evident that the identity of the species of this genus cannot be based on any one set of characters either morphological, physiological, or pathogenic. It also becomes evident that in order to understand plants which are so sensitive and are so difficult to separate into specific groups by their morphology alone, the worker must be thoroughly acquainted with all their specific reactions under controlled conditions.

Structure of the Zoöspores of P. palmivora.—The size of the spores of the plants studied corresponds to the measurements given by Butler

in his description of the species (Mem. Dept. Agr. India Bot. Ser. I, 5: 1-60. 1907). The spores are kidney-shaped or ovoid with a flattened space on one side where the cilia appear. This is known to be the characteristic shape of the zoöspores in this group. There are, however, many spores which vary all the way from kidney-shaped to a nearly spherical structure, usually, however, with a somewhat flattened dent at the insertion of the cilia. At optimum conditions the cilia are two in number and usually have their insertion in a definite chromatic body, the basal granule, which in turn is connected with the nucleus (Pl. XXXII, fig. 42). In some of the spores the cilia appear to be connected directly to the nucleus; in such cases it is impossible to differentiate the basal granules (fig. 44). In certain spores only one cilium could be found to have definite nuclear connections, the other having its origin in a basal granule; the latter was somewhat larger than that found where the basal granule was connected with the nucleus by a definite strand. There are also occasionally triciliated spores in which the basal granule of one cilium is definitely connected with the nucleus while the other two cilia have their insertion in separate basal granules, with which there was no apparent connection with the nucleus or with each other (fig. 43). The formation of extra cilia and of cilia with basal granules entirely separated from the nucleus, is interpreted as being the result of the basal apparatus at one pole of the nucleus becoming separated from the nucleus early in the process of cilia formation. This is probably the result of an earlier condition similar to that described for the early stage of the spores of *Aphanomyces euteiches* and shown in figure 34 *a*. This triciliated condition with two of the cilia inserted on basal granules entirely separate from the nucleus would seem to indicate more definitely that the basal granules are directly responsible for cilia formation, and possibly also for the later control of the activities of these organs.

The cytoplasm of these spores is very finely granular. The more dense portion surrounding the nucleus is quite limited in some cases as in the spore shown in figure 44. In other spores this central zone of cytoplasm is more extensive (fig. 42). There are occasional larger, more densely stained granules scattered through the cytoplasm. These granules are more prevalent in the central than in the peripheral cytoplasm. The peripheral cytoplasm is less dense and is filled with many small vacuoles which give to this part of the cell a rather loose appearance.

GENERAL DISCUSSION

Influence of External Conditions

The importance of the influence of external conditions on the ability of various species of the Oömycetes to produce the different phases of their life cycles has been shown by a number of investigators including Klebs (27), Kauffman (25), Pieters (35), and others. Klebs was the first to show that zoöspore formation among the Oömycetes is initiated when a well-nourished

mycelium suddenly experiences a lack of food, as, for example, when it is thoroughly freed from an external food supply and then transferred to pure water. It is evident from the work reported in this paper that, even though a plant may have a wide range of conditions under which zoöspore formation is initiated or partially stimulated, there are definite narrow limits of temperature and aëration, and the maximum number of zoöspores will be formed only within such limits. It is seen, further, that the typical zoöspores are produced only from a vigorous, well nourished mycelium from which the food has suddenly been thoroughly removed. In other words the removal of the food from the young active mycelium sets in motion the potential ability to form sporangia and zoöspores while the completion of this process, especially in a manner typical of the species, depends on favorable conditions of temperature and aëration. Temperatures above the optimum and poor aëration, conditions which are unfavorable to the process, often result in incomplete cleavage of the protoplasm in the sporangium. Such conditions may completely inhibit zoöspore formation or cause the formation of giant spores. In a preparation which was well-aërated at the beginning, loss of oxygen occurs more rapidly at the higher temperatures. This is due to the fact that, at these temperatures the oxygen in the preparation is used up, and the products of metabolism are liberated faster than at lower temperatures. The liberation of carbon dioxide is frequently used as an index to these activities. Kostychev (28) has stated that "the amount of carbon dioxide given off increases regularly with the gradual increase in temperature, finally reaches a maximum value and remains at the same level until the death of the plant from the high temperature (at about 50°)." It is apparent then that when plants with a low optimum for zoöspore formation, as for instance *Blastocladia Pringsheimii* or *B. globosa*, are studied under ordinary room temperatures which range from 6° to 10° C. higher than the optimum, the zoöspores are quite likely to be atypical. In plants where the optimum temperature for this process falls near to or within the range of ordinary room temperatures as in *Saprolegnia monoica* var. *glomerata* or *Isoachlya paradoxa*, giant spores due to incomplete cleavage are only occasionally liberated and then only in the interior of the mount where the sporangia are smothered. This is more likely to occur when too much mycelium is used in the preparation of the mount, and is interpreted as being due to poor aëration in this part of the mount.

Cilia Formation in Uniciliate and Biciliate Groups

The question naturally arises as to just what are the cytological, physiological, physical, or chemical differences between the uniciliate and the biciliate groups at the time of zoöspore formation. One may ask, are there any observable details that may be pointed out to account for the fact that in one group the spores are normally biciliate while in the other

group we find the uniciliate condition to be characteristic of the motile spores? In the case of the second swimming stage of the diplanetic species, during and immediately following the process of freeing themselves from the resting cysts, a marked increase in size is noticed. This is brought about by the rapid absorption of water along with the formation of many vacuoles in the peripheral part of the cytoplasm. The cilia are formed by these spores during the period when water is being absorbed with an accompanying increase in size. It is not definitely known, on the other hand, at what exact stage the cilia are formed in the maturation of the spores in the sporangia. Davis (15) in his study of the young zoösporangium of *Saprolegnia* has shown that the nuclei are not active before or during the cleavage of the protoplasm into uninucleate spore initials and are not ciliated at this time (Davis, 15, figs. 30-35). There is further evidence that the cilia are often not completely matured when the spores are liberated from the sporangium (Couch, 13). The writer has shown that cilia formation is initiated in the sporangium of *Achlya* after cleavage of the protoplasm into spore initials (page 526). However, the cilia are not formed immediately after cleavage in this biciliate group of plants but probably immediately prior to the liberation of the spores from the sporangium.

Barrett (2) in his study of *Allomyces* has shown that the nuclei are evidently concerned in the early process of cilia formation during or soon after cleavage has taken place. In his figure 31, it will be noted that the beak-like extensions of nuclei have formed and are in some cases in contact with the newly formed plasma membrane. Speaking of this figure Barrett says, "Fig. 31 represents a part of a section of a sporangium in which the segmentation is almost complete. . . . The limiting surfaces of the spore masses in a number of cases have separated. . . . Apparently contraction has taken place, which would indicate that the mature spores occupy less space than the original masses of protoplasm from which they were formed." It seems apparent that cilia formation in this species is initiated during or immediately following the cleavage process and is probably immediately preceded by nuclear division, since Barrett has shown that nuclear division does take place in the sporangium prior to cleavage. Jahn (23) in her studies of nuclear division and cilia formation in the zoöspores of *Stemonitis flaccida* Lister, has shown that cilia formation accompanies nuclear and cell division in this case. It thus becomes apparent that, in the spores of at least a number of the species of the uniciliate groups of fungi, cilia formation accompanies or follows closely nuclear and cell division. In these cases a single cilium is initiated or formed at each pole of the mother nucleus, which finally results in the presence of a single cilium for each of the daughter cells or zoöspores of the species.

On the other hand, in certain fungi which have under optimum conditions biciliate zoöspores, it is known that cilia formation does not accompany or closely follow nuclear or cell division. In the spores in the second

swimming stage of species of *Saprolegnia*, *Achlya*, and *Aphanomyces* cilia formation is preceded or accompanied by nuclear activity and spindle formation. The cilia are formed as a result of activities centered in certain granules which occupy or formerly occupied a position at the poles of the nucleus. During this process it should be noted that instead of cell division there is a marked increase in size of the protoplast accompanied by the absorption of water and the formation of many small vacuoles in the peripheral cytoplasm. This it will be noted is exactly the opposite of what takes place during the process of cleavage of a mass of protoplasm, as has been shown by Hofmeister (21) who pointed out that the division of the protoplasm in the process of cleavage is accompanied by loss of water, increased density, and reduction of volume. Harper (18) states that "in the gradual shrinkage and condensation of the spore-plasm the loss of water might be least in the neighborhood of the nuclei and that thus a determining factor in the orientation of the cleavage surfaces would be introduced." Swingle (42) says that "the most probable explanation the writer has found for the mechanics of cleavage is on the basis of local contractions of the cytoplasm, somewhat comparable to the phenomenon exhibited in the naked protoplasm of amoebae and pseudopodia." It is apparent then that cilia formation in the spores of the uniciliate and of the biciliate species of fungi takes place under entirely different internal conditions. The initiation of cilia formation takes place during very early stages of spore formation in the uniciliate group. In the biciliate group this process is delayed until later when greater nuclear activity develops; the latter is sufficient to bring about cilia formation but the conditions present do not permit of further cell division. Back of all this, of course, lies the different genetical make-up of the species in the two groups.

Structure of the Basal Apparatus and its Relation to the Nucleus

The status of our knowledge concerning the basal apparatus as found in various ciliated cells has been adequately discussed in the work of Wilson (45) and of Sharp (41). The writer therefore will not review this material, but refers the reader to those authors for the general discussion of points bearing on this subject.

Hartman (19) has shown that in *Eudorina elegans* the pointed end of the nucleus to which a "centriole" is attached, touches the plasma membrane and then retreats leaving behind a double body from which the cilia grow. This description of the formation of the cilia in *Eudorina elegans* seems to be the nearest approach, of anything described in the literature, to what happens during cilia formation in the zoöspores of the fungi studied by the writer. In the zoöspores, especially of the second swimming stage of species of *Achlya*, *Aphanomyces*, and *Saprolegnia*, the writer has shown, however, that both poles of the nucleus, with their attached or included granules, approach very near to or actually touch the plasma

membrane; they may then withdraw from the membrane leaving there one or more granules. At points near these granules we find the cilia appearing, first as pseudopod-like projections, which later are matured into active cilia. The question now arises, are these basal granules, which seem to control the development of cilia in the zoöspores of the Oömycetes, homologous with the basal apparatus of the protista and those found in the spermatogenesis of some animals and plants? Wilson (45) has said that "the relations between these various basal structures have not yet been completely elucidated."

It would seem, however, that the basal apparatus in these fungi is very definitely of nuclear origin. In the development of the spores of the second swimming stage of *Achlya conspicua*, *Aphanomyces euteiches*, and *Saprolegnia monoica* var. *glomerata*, it is shown that these granules, often three in number for each cilium, are first found occupying positions near the poles of the crescent-shaped nuclear apparatus as the spore prepares for cilia formation. If the granules at one pole of the nucleus become completely separated from the nucleus early in the process of cilia formation, but remain in close proximity with each other, they may under certain conditions remain separate from the nucleus and have complete control of the development and function of one cilium (*Aphanomyces*, fig. 40). If, however, these granules become somewhat more separated from each other, a separate cilium may be developed from each granule (*Phytophthora*, fig. 43). It is apparent, then, that even though these basal granules are shown to be of nuclear origin they may develop and control cilia after, in so far as one can see, they have been completely separated from the nucleus. This would seem to show that the cilia of the spores in these fungi may have their origin and may be controlled by differentiated parts of the nuclear material and not by the entire organ.

In the majority of cases of normal spore development of the second swimming stage both cilia are found to remain attached to the nucleus. They or one of them may have migrated away from the pole or poles of the nucleus so as to occupy a position on one side of that organ. This position probably has something to do with the usual shape of the spores at this stage. There are definite, more dense chromatic bodies inside the nucleus in which the strands (rhizoplasts) running from the basal granule to the nucleus have their origin. The writer is inclined to believe that normally but one granule is dropped from each tip of the nuclear apparatus, and that the remaining granules near the tip form this denser chromatic body. When the basal granules along with their cilia have migrated fairly close together these denser chromatic bodies often seem to fuse forming a larger somewhat curved body (*Rhipidium*, fig. 13). In the first swimming stage of the diplanetic species this chromatic body at the insertion of the rhizoplasts in the nucleus is often somewhat V-shaped. In very favorable material a granule may be found near each leg of the V to which the rhizo-

plast is attached. The base of the V is evidently formed by the fusion of two or more granules which formerly formed the extreme base of the rhizoplast from each cilium (*Saprolegnia*, fig. 16).

It is therefore apparent that, in the zoöspores of the fungi here discussed, the basal granules are of nuclear origin. The basal apparatus is composed of several granules, usually three in number, two of which usually remain within the nucleus and form the denser chromatic body in which the connecting strand or rhizoplast has its insertion in the nucleus. This body under certain conditions may apparently fuse with the similar body of the basal apparatus of the other cilium forming the larger body described above. The other granule forms the basal granule proper, or "blepharoplast," at the base of the cilium.

The Encysted Spore Stage of Diplanetic Species

A number of interesting questions arise in connection with the encysted stage of the zoöspores of the diplanetic species of the Oömycetes. Why should such encystment be interpolated between the two swimming stages? There is also the question of the relation of the spores of the second swimming stages of these plants to spores of similar morphology and cytology of monoplanetic species. It is evident that an adjustment of the nuclear apparatus takes place during or immediately following the encystment. This results in morphological changes which are present in the second swimming stage. There is also a marked change in the physiological reactions of the spore of the second swimming stage and this is probably controlled by this nuclear adjustment. The spore is now able to function as a reproductive body. It is now able to absorb food and initiate the process of growth resulting in the production of a vegetative mycelium. Apparently it could not accomplish this process before this nuclear and the accompanying physiological adjustment had taken place. The behavior of the zoöspores of some related groups which have but one swimming stage and which resemble in structure and function the spores of the second swimming stage of the diplanetic species, may be explained by the fact that this nuclear adjustment in the monoplanetic species has taken place before liberation from the zoösporangium. It is not difficult to believe that the *Achlya* type of diplanetic spores, where cilia formation in the first swimming stage is initiated but usually not completed, and *Aphanomyces*, where there is no indication of cilia formation in this stage, are but a step removed from the condition where this nuclear and physiological adjustment takes place in the sporangium.

Ciliation a Constant Character

There is one other important relation brought out by this work which should be emphasized. The writer refers to the importance of the ciliation of the zoöspores in connection with the classification and possible phylo-

genetic connections of the group. The writer (12) has pointed out in his studies of the genus *Blastocladia*, that "the cilia are constant in number and position when allowed to develop normally under conditions most favorable for zoöspore formation." The present work makes it possible to apply this statement to a much larger group of the Oömycetes if not to the entire sub-class. Even "giant zoöspores"⁴ show the characteristic number of cilia in relation to each nucleus when properly stained and cleared. It is apparent then that ciliation of the zoöspores of the Oömycetes is a stable character when the spores are studied under favorable conditions and properly stained. The number of cilia of the zoöspores may now be recognized as it has been by some botanists as one of the fundamental characteristics of these plants. It is a useful character in connection with the classification of the larger groups of the Oömycetes, and in any considerations dealing with the phylogeny of the group.

SUMMARY

1. In order to obtain zoöspores of the Oömycetes at will, in abundance and in normal condition, so that they may be studied in any stage of development desired, optimum conditions of temperature and aëration must first be thoroughly controlled by the experimenter.

2. For each species there is a certain definite set of conditions under which it is capable of forming most abundantly perfect zoöspores typical of the species. Any single factor or set of factors, such as temperature, aëration, and food supply influences the formation of sporangia or of zoöspores.

3. The optimum temperatures for the formation of zoöspores were determined in the following species: *Allomyces arbuscula*, between 25° and 27° C.; *Saprolegnia monoica* var. *glomerata*, very near 26° C.; *Isoachlya paradoxa*, between 23° and 25° C.; *Achlya conspicua*, between 25° and 26° C.; *Aphanomyces euteiches*, from 26° to 28° C.; *Apodachlya brachynema*, between 15° and 19° C.; *Rhipidium europaeum*, between 16° and 18° C.; *Phytophthora cactorum*, *P. erythroseplica*, *P. parasitica*, *P. terrestris*, and *Phytophthora* sp. between 25° and 27° C.; *Phytophthora palmivora*, between 23.5° and 25.5° C.

4. A technique has been developed by which zoöspores may be so stained as to demonstrate the basal apparatus of the cilia and its connection with the nucleus. This technique involves killing with fumes of osmic acid, staining with crystal violet, and drying of the preparation (over sulfuric acid), followed by the usual procedure of clearing and mounting.

5. The zoöspores of the species studied all have a definite basal granule

⁴ In a paper which came to hand after the present paper was completed, Hans Kniep (Ber. Deutsch. Bot. Ges. 47: 199-212, 1929) has shown, in his study of *Allomyces javanicus* n. sp., that "giant zoöspores" occur in his species of *Allomyces* similar to those reported by the writer (12) for *Blastocladia globosa* and *B. pringsheimii* and in this paper for *Saprolegnia monoica* var. *glomerata*; both Kniep and the writer have interpreted the phenomenon in the same way.

(blepharoplast) at the insertion of each cilium. This granule is connected with the nucleus by a definite strand (rhizoplast).

6. The cilia develop as outgrowths from the region of a basal granule which has reached a position near the plasma membrane as a result of nuclear activity.

7. The basal apparatus is always of nuclear origin and seems to be composed of several chromatic bodies, one of which forms the basal granule proper (blepharoplast). The remaining granules of the basal apparatus are normally retained or drawn back into the nucleus and usually form the tip or beak-like part of that organ during zoöspore activity.

8. When the zoöspores of either swimming stage come to rest, the basal granule or granules, as the case may be, migrate back to the nucleus and apparently become part of that organ.

9. The chromatin of the nucleus in the mature active zoöspores of these fungi is arranged in irregular masses in the peripheral region. In some spores a larger central mass of chromatin may be distinguished near the center of the nucleus.

10. Temperature conditions away from the optimum for a species have been shown to be the cause of certain abnormalities hitherto described in the literature, as for example variations in the number of cilia or the presence of "giant" spores.

11. The results obtained by the study of such a large number of examples from every group of the Oömycetes have emphasized the phylogenetic value of the cilia because of their constancy in a genus.

The studies reported in this paper were completed in the Botanical Laboratory of the University of Michigan under the direction of Dr. C. H. Kauffman who suggested the problem and to whom the writer wishes to express his appreciation for the many helpful suggestions made and the keen interest shown during the progress of the work and the preparation of the manuscript.

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DESCRIPTION OF PLATES

All drawings were made to the same scale, with the aid of a 2 mm. oil immersion objective, a 12 × compensating ocular, and a camera lucida.

PLATE XXX

FIGS. 1-6. *Allomyces arbuscula*

FIG. 1. Young zoöspore with immature cilium, note basal granule with 3 smaller granules connected with it, and situated adjacent to the nucleus. The chromatin of the nucleus is distributed around the periphery and in a central mass which in turn is connected with the basal apparatus. All of the deeply stained granules in the cytoplasm are situated in the distal region of the spore.

FIG. 2. A somewhat older spore in which some of the deeply stained granules have been moved toward the ciliated end of the spore. The three-parted basal apparatus is also evident in this spore.

FIG. 3. The granules of the basal apparatus nearest the nucleus have migrated together, apparently forming a single larger body which now forms the tip of the nucleus. The dense mass of cytoplasm in front of the nucleus is beginning to be crowded back into a rather regular oval mass almost enclosing the nucleus.

FIGS. 4-6. Different stages in the orientation of cell structures due to activity of the spore; note change in position of deeply stained cytoplasmic granules in spores 3, 4, 5, and 6.

FIGS. 7-10. *Apodachlya brachynema*

FIG. 7. First swimming stage, note individual basal granules with separate nuclear connections.

FIGS. 8-10, second swimming stage. FIG. 8. The basal granules are apparently fused, but separate nuclear connections may be seen. The peripheral cytoplasm shows many small deeply stained granules.

FIG. 9. The separate basal granules for each cilium may be distinguished in this spore.

FIG. 10. Spore in which the distal part of the nucleus has not become completely rounded off following the formation of the cilia.

FIGS. 11-13. *Rhipidium europaeum*

FIGS. 11 and 12. Spores in which the cilia remain attached to opposite parts of the nucleus before migrating to one side; note regular arrangement of the vacuoles in the peripheral cytoplasm.

FIG. 13. Side view of a spore in which the cilia along with each basal apparatus have become oriented on one side of the nucleus.

FIGS. 14-24. *Saprolegnia monoica* var. *glomerata*

FIGS. 14-17, first swimming stage. FIG. 14. Large spore with the basal granule for each cilium well differentiated. The nucleus is nearly round with a central mass of chromatin which is connected with the basal apparatus. The cytoplasm is finely granular and more dense in the central region and more vacuolate towards the periphery; occasional deeply stained cytoplasmic granules may be seen.

FIG. 15. A smaller spore with the nucleus somewhat over-stained. A vacuole is evident in the distal part of the nucleus.

FIG. 16. Spore with the strands connecting the basal granules with the nucleus crossed, showing their origin in this case to be in separate granules inside the nucleus, these granules being connected each with one leg of a V-shaped mass of chromatin. The central cytoplasm shows many deeply stained granules.

FIG. 17. A giant spore with two nuclei each characteristically biciliate.

FIGS. 18-24, second swimming stage. FIG. 18. Bristle-like projections of the cytoplasm shortly after initiation of cilia formation, somewhat over-stained.

PLATE XXXI

FIG. 19. A slightly later stage with the nucleus near the surface of the spore. The basal granules may be seen near the tips of the nucleus.

FIG. 20. The young cilia have touched and adhere to each other at the tip.

FIGS. 21, 22, and 23. Relatively young spores. Note that the base of each cilium is enclosed in a cytoplasmic matrix; the nuclear connections (rhizoplasts) are less evident at this stage than in spores which have been active for a longer time.

FIG. 24. Side view of spore which has been active for considerable time. Note that connections between basal granules and nucleus are quite distinct, each basal apparatus in this spore has a separate attachment in the nucleus. The cytoplasm is divided into a central more dense and a peripheral more vacuolate region.

FIGS. 25-32. *Achlya conspicua*

FIG. 25. Spore initials showing crescent shaped nuclei, *a* with one pole, *b* and *c* with both poles with included granules approaching the plasma membrane.

FIG. 26. Two of a group of spores before emerging from a sporangium showing connecting strand.

FIG. 27. Spore which was not attached to others in sporangium which was in process of being emptied. Note initiation of cilia formation with granules near the tip of the finger-like projections.

FIG. 28. Spore just liberated from sporangium. Note two short cilia, one of which, *a*, lies near an old connecting thread, *b*; the nucleus appears to have been folded together with each basal apparatus remaining attached to each tip.

FIG. 29. *a*, young cilium has been absorbed but basal granules are still near surface of spore; *b*, basal granules apparently pulling plasma membrane slightly toward nucleus; *c*, basal granules free from plasma membrane have migrated almost to the nucleus.

FIG. 30. Spore going into encystment. Note nearly spherical nucleus.

FIG. 31. Young spore of second swimming stage, cilia nearly ready for use in locomotion. Note attachment of tips to piece of debris, and waves in the cilia when the spore was killed.

FIG. 32. *a*, spore of second swimming stage which has been active. The basal granules appear to be fused forming a rod-shaped body which seems to be attached to the nucleus by a single strand. Note two distinct zones of cytoplasm and the presence of many deeply stained cytoplasmic granules; *b*, similar to spore (*a*) except that basal granules are separate and distinct.

PLATE XXXII

FIGS. 33-41. *Aphanomyces euteiches*

FIG. 33. Spore which has just emerged from the sporangium, the connecting threads are still evident.

FIG. 34. *a*, spore just out of encystment. Note granules which have been dropped from one tip of the nucleus, and included granules in other tip; *b*, spore with 3 granules in each tip of the nucleus. Note a single granule at each extreme tip or pole of the nucleus; this is evidently the young basal granule proper (blepharoplast); *c*, spore showing nucleus with two leg like projections. Note granule in tip of each.

FIGS. 35, 36, 37, and 38. Various stages in the development of cilia a short time after the spores have emerged from the encysted stage. Note the loop-shaped form of the young cilia, with granules variously distributed at first, and later mostly concentrated at the base of the cilium. In figs. 37 and 38 the cilia are approaching maturity, and are nearly free from the debris to which they cling.

FIG. 39. Spore with cilia still attached to each other, but evidently in last stages of freeing the tips of the cilia.

FIG. 40. Mature spore in which the one basal apparatus was evidently completely dropped by the nucleus, probably as in fig. 34*a*.

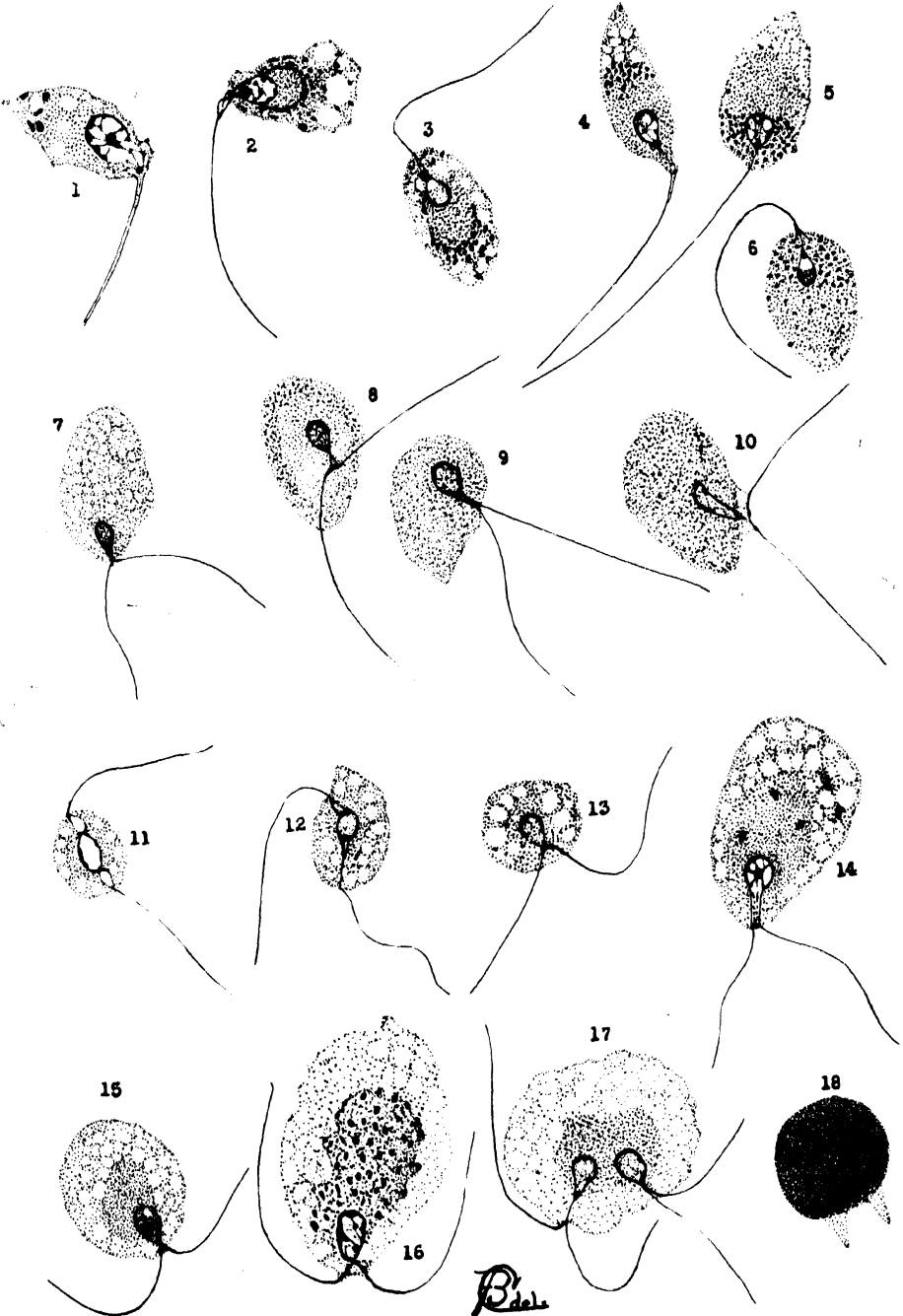
FIG. 41. Active spore with cilia connected with different parts of the nucleus. Note basal granules, and denser body inside the nucleus at the insertion of each connecting strand. The cytoplasm is finely granular, somewhat more dense towards the center near the nucleus.

FIGS. 42-44. *Phytophthora palmivora*

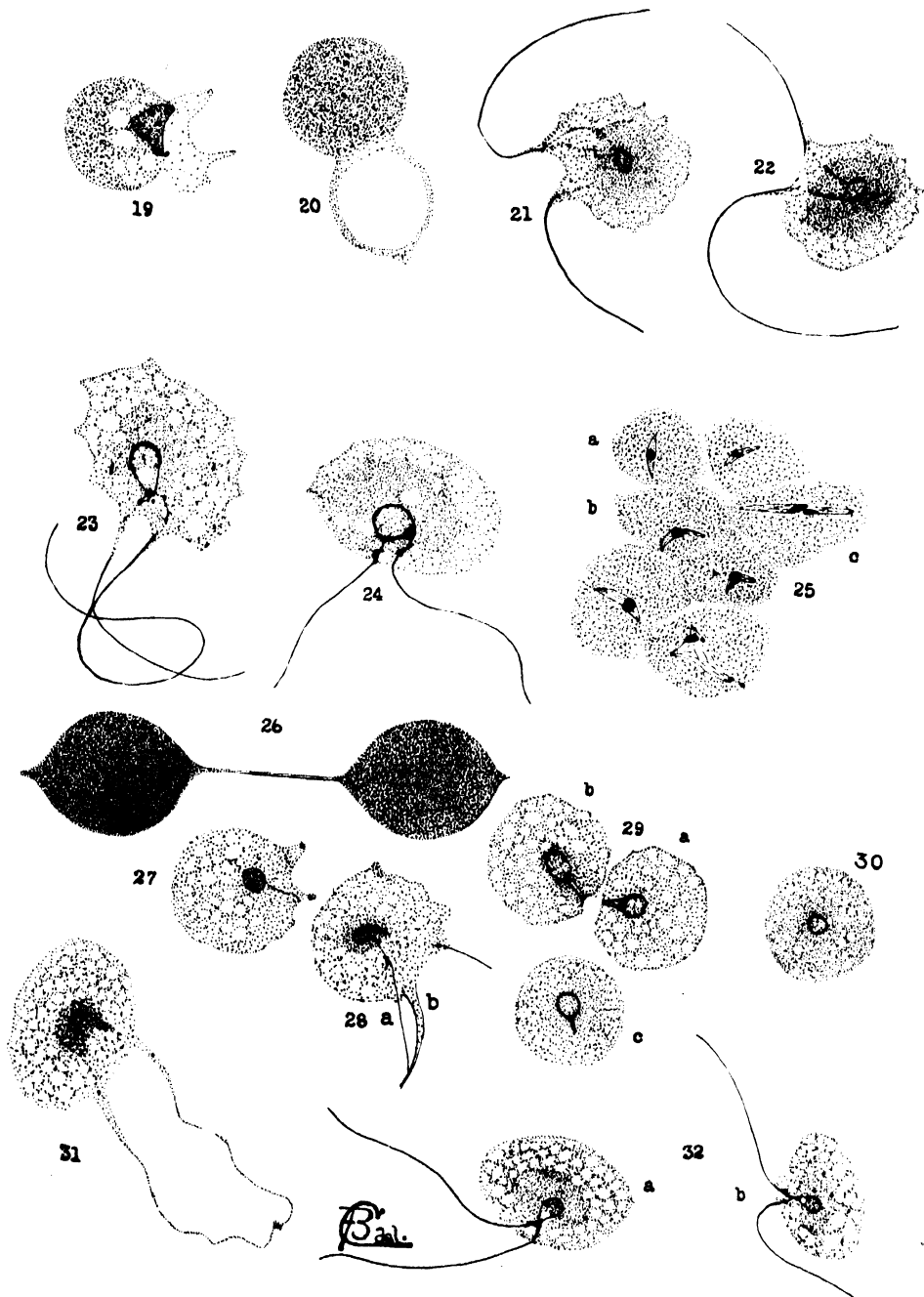
FIG. 42. An active spore showing separate basal granules with connecting strands inserted in the same dense chromatic body in the nucleus. The cytoplasm is finely granular and more dense toward the center and more vacuolate toward the periphery.

FIG. 43. Spore with three cilia, two of which are apparently separate from the nucleus, evidently the result of one basal apparatus becoming completely separated from the nucleus.

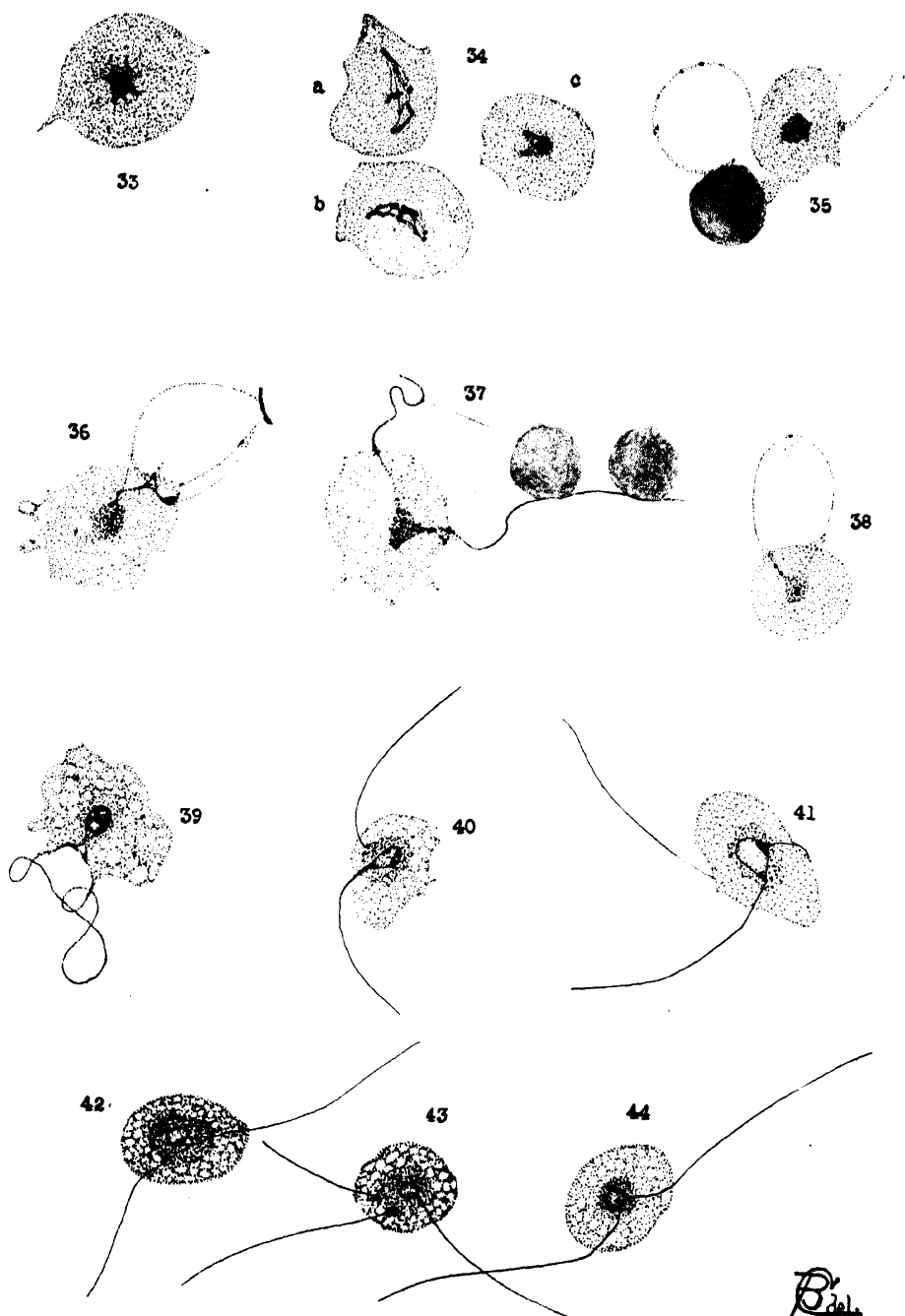
FIG. 44. Spore in which the cilia seem to be attached directly with the nucleus; the basal granule is evidently near the surface of the nucleus.



COTNER: ZOÖSPORES OF OÖMYCETES



COTNER: ZOÖSPORES OF OÖMYCETES



COTNER: ZOOSPORES OF OOMYCETES

A PITYOXYLON FROM YELLOWSTONE NATIONAL PARK

HENRY S. CONARD

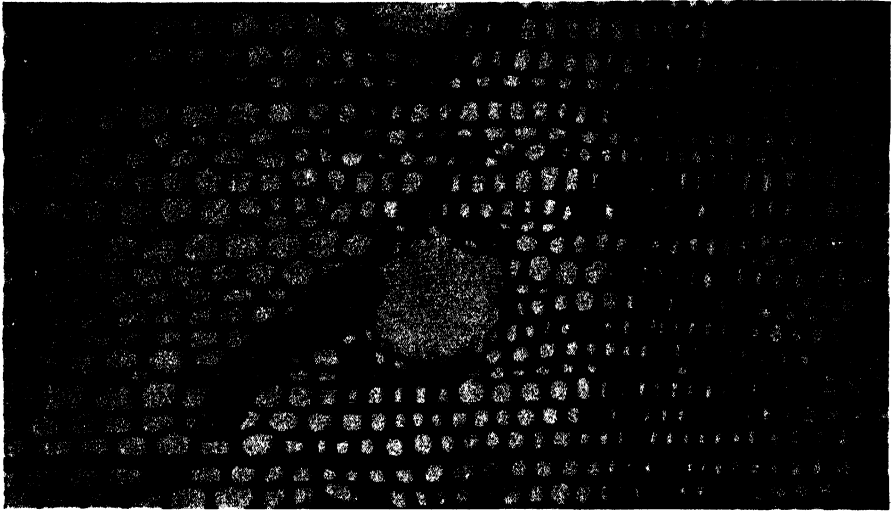
(Received for publication February 3, 1930)

The Yellowstone National Park is the product of the activities of three major volcanoes. One of these, the Sherman Volcano, had its center between Mt. Washburn and Prospect Mt. directly south of Tower Fall and Camp Roosevelt, in the northeastern part of the Park. This volcano buried up a bit of Eocene vegetation in its earlier days, continued active throughout the Miocene, and ended with the Pliocene basalts (2). Buried in the breccias of the volcano we find the countless petrified trees of Specimen Ridge and vicinity. Many standing trunks are exposed now by Pliocene and Pleistocene erosion, and many more petrified trees have been broken to pieces and strewn all over the valley of the Lamar River. Pieces of petrified wood are found everywhere, sometimes as angular wood-like fragments, often as water-worn pebbles. In the summer of 1925 a glacial boulder of petrified wood eighteen inches in diameter was found at a 7800-foot elevation on Crescent Hill. Standing trunks broken off at ground level occur on Prospect Mt. up to a 9000-foot elevation.

None of these localities or specimens has been adequately investigated. The only publications giving any details are those of Felix (1), and Knowlton (2, 3). Knowlton describes only one species under the generic name of a living plant, *Sequoia magnifica*. This he says is not really distinguishable from *S. sempervirens*. The similar fossils "above Yancey's Ranch" (the petrified tree of guide books) he cautiously calls *Cupressinoxylon*. His Alderson Pine and Amethyst Pine are called *Pityoxylon Aldersoni* and *Pityoxylon amethystinum*. I have seen the type specimens of the above species on Specimen Ridge and above Yancey's many times, and have examined sections of two of them.

In the summer of 1924 I obtained for the Yellowstone Park Museum a waterworn pebble of petrified pine which I brought home for precise identification. It was sent to Professor Knowlton at the Smithsonian Institution. There a chip was broken off and ground down, giving transverse, radial, and tangential sections of exquisitely perfect preservation. All former collections have been very obscure in the radial section. At the suggestion of Professor Knowlton I am therefore offering the following emended description of *Pityoxylon amethystinum* Kn.

Transverse section, text figure 1.—Annual rings very distinct, 2 to 6 mm. wide, the summer wood being about one half the width of the spring wood of the same year, that is, the summer wood occupies about one-third of



TEXT FIG. 1. Transverse section. Spring wood (left) merging into summer wood (right) of the same year. Large resin canals.

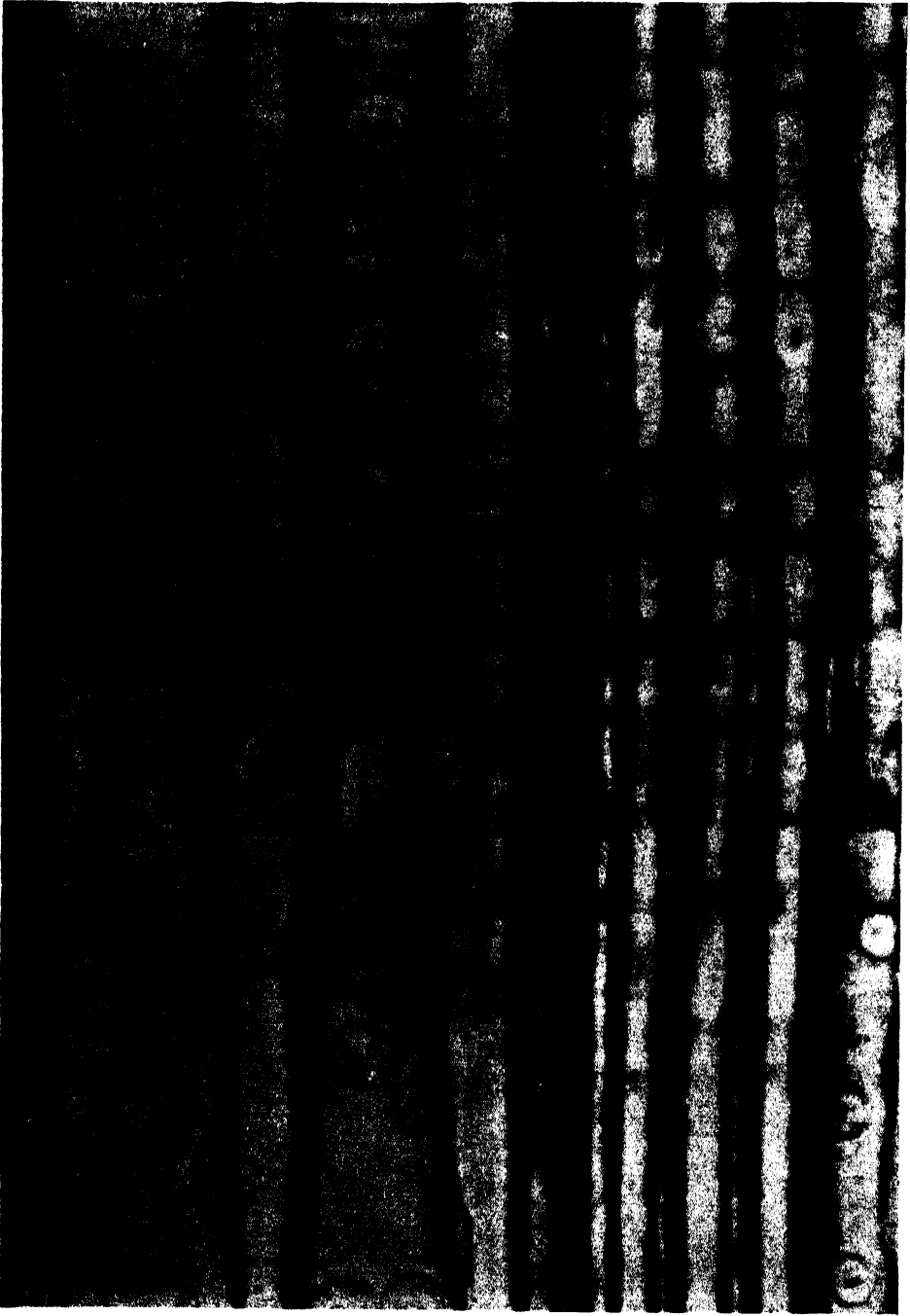


TEXT FIG. 2. Tangential section in summer wood. Longitudinal and transverse resin canals. Uniseriate and fusiform rays.

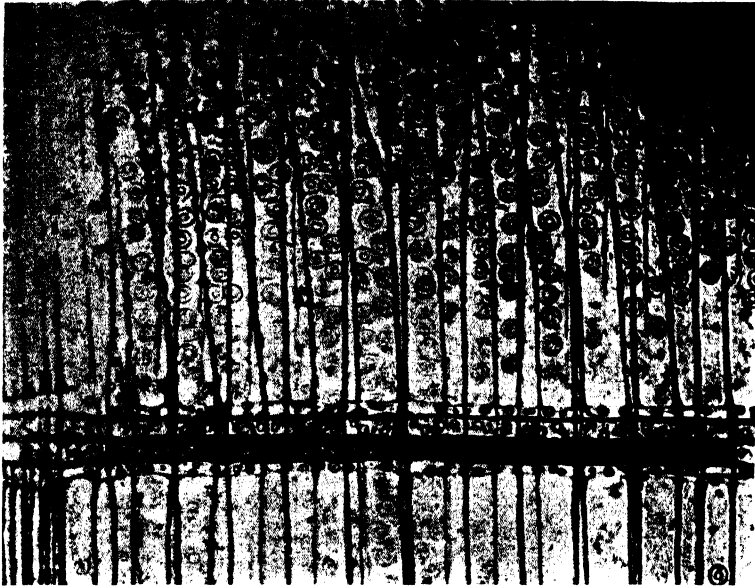
the annual ring. Boundary of the annual ring very distinct. Boundary between spring and summer wood of one year so abrupt as to simulate the end of a year's growth. Cells of spring wood large, 0.04–0.065 mm. in radial diameter by 0.02–0.055 mm. in tangential diameter, thin walled, bordered pitted on radial walls, rectangular to hexagonal. Cells of summer wood small, rectangular, 0.02–0.03 mm. radially (lumen half of these dimensions), by 0.025–0.05 mm. tangentially, the walls thick, not pitted at all. Resin canals large, 0.14 mm. across, in late spring and summer wood of wide rings, confined to summer wood in narrow rings; canal surrounded by large, more or less disintegrated thin walled epithelial cells and irregular parenchyma; tyloses rare and only partial. Rays separated by 3 to 25 wood cells, thin, straight, without outwardly bulging walls; entire ray solid black. There are single tracheids or groups or rows in the spring wood whose dark color and content suggests the resin filled tracheids of various authors (Torrey, 5), but such cells could not be identified in the longitudinal sections.

Tangential section, text figure 2.—Rays numerous. Uniseriate rays 1 to 15 cells high, mostly 10 to 12; cells isodiametric to oval, 0.015–0.02 mm. wide by 0.01–0.025 mm. high, thick walled, not showing pits, all alike except marginal cells (ray-tracheids), without contents, or occluded. Marginal cells ovate-triangular, acute, twice as high as wide, e.g., 0.015 mm. by 0.03 mm. Fusiform rays numerous, the median resin canal with its epithelium occupying the entire width of the ray; cells above and below canal 3 or 2, then 2, then 1; total width of ray 0.04 mm. Wood cells very distinct, without pitting (summer wood). Longitudinal resin canals sheathed with large, thin walled, rectangular epithelial cells.

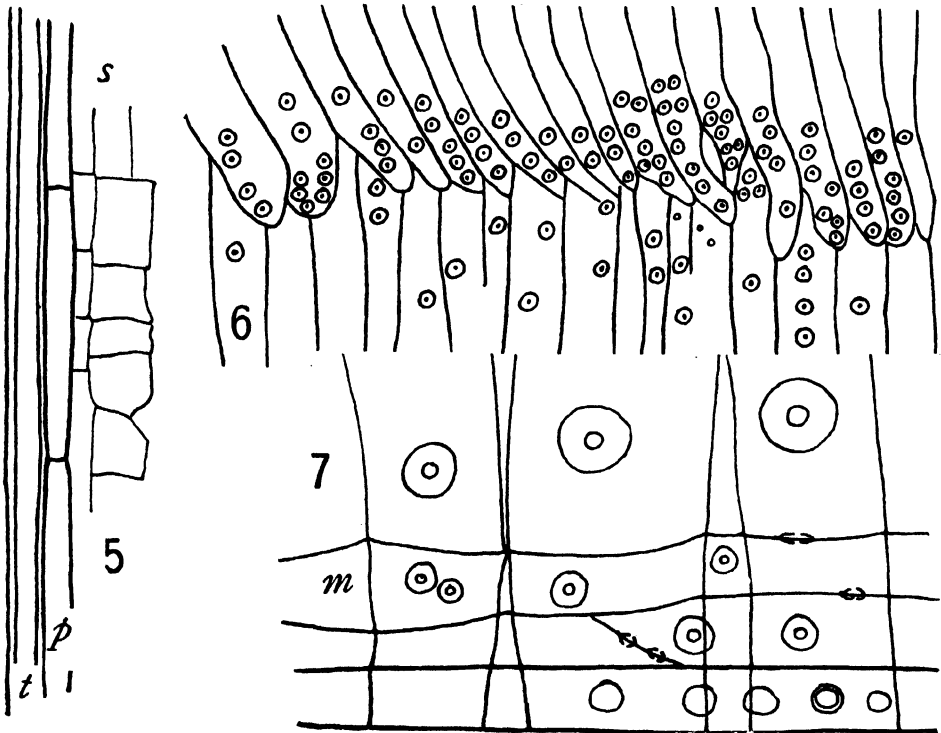
Radial section, text figures 3, 4.—"The radial section of nearly all woods from the Yellowstone National Park is more or less obscure. The one under consideration is no exception to this rule" (Knowlton, 1899, p. 764). Our specimen is almost as perfect as a recent wood. Fiber cells of spring wood 0.072–0.087 mm. wide; border pits large (0.022 mm., with circular pore 0.008 mm.), conspicuous and perfect, in one wavering row, close together near ends of cells, scattering and often absent for half a millimeter or more midway of cells; rarely two pits side by side, though in one place there are 3 pairs in series (text fig. 3). Bars of Sanio very plain, each bar appearing as a pale line between two fine darker lines. Walls of cells perforated with minute scattered pores, smaller than the pore of a pit; these pores occur on plain walls, on margins of bordered pits, on ray-tracheids, and may be due to some agency operative after the death of the tree. Ends of cells mostly slightly curved, with blunt rounded tips, many cells ending at one level (text fig. 6); some cells end straight and tapering to a point. Longitudinal resin canals lined with large, rectangular, thin walled epithelial cells. Between these and the wood fibers are wood parenchyma cells, long-rectangular, with medium thick walls (text fig. 5).



TEXT FIG. 3. Radial section. Tangential pitting between spring and summer wood of successive years. Large uniseriate and biseriate bordered pits, and bars of Sanio in spring wood. Bordered pits appear in sixth from last summer tracheid.



TEXT FIG. 4. Radial section showing ray.



TEXT FIG. 5. Margin of resin canal; *p*, parenchyma; *s*, secretory cells; *t*, tracheid.

TEXT FIG. 6. Ends of tracheids.

TEXT FIG. 7. Margin of ray; *m*, ray-tracheid.

Rays numerous. Marginal cells in one or two rows, with wavy outer wall, the peaks corresponding with walls of wood cells; walls thin (0.001–0.002 mm.), smooth in both spring and summer wood, with small bordered pits, the pores round or oval, one or two pits to a wood cell; end walls oblique, with one or two small bordered pits (text fig. 7). Walls of true ray cells medium thick (0.003–0.005 mm.), the lateral walls with two (1 to 3) small rounded simple or narrowly bordered pits adjacent to each wood cell. End walls transverse or oblique, sometimes appearing simply pitted, but this detail could not be assured; rarely indications of such pitting appear on upper and lower walls of ray cells. Adjacent to the longitudinal resin canals, the ray cells have a network of many simple pits. At one point in my section a wood cell on the boundary between summer of one year and spring of the next shows tangential pitting (text fig. 3). The wall in question is of the thickness of a spring wood cell. Other than this no tangential pitting has been found.

Our specimen is a perfectly typical *Pityoxylon* as defined by Torrey (5). Its identity with Knowlton's *P. amethystinum* is reasonably certain, as shown by table 1.

TABLE 1. *Comparison of the Material under Investigation with Pityoxylon Aldersoni and P. amethystinum*

| | <i>P. Aldersoni</i> | Our Specimen | <i>P. amethystinum</i> |
|--------------------------------|---------------------|------------------------|------------------------|
| Width of annual ring..... | 6–9 mm. | 2–6 mm. | 3–8 mm. |
| Resin canals in..... | summer wood only | late spring and summer | late spring and summer |
| Cells between rays..... | 3–10 | 3–21 | 3?–25 |
| Rays in tangential section.... | numerous | abundant | abundant |
| Height of rays..... | 2–30 cells | 1–15 cells | 2–12 cells |
| | av. 15 | av. 8 | av. 6 |
| Fusiform rays..... | rare | numerous | numerous |

The occurrence of this specimen, which agrees so closely with *P. amethystinum* and is clearly distinguishable from *P. Aldersoni*, provides further evidence that the two species are really distinct. On this point Knowlton says, p. 765: "This species is very closely allied to the one preceding, *P. Aldersoni*, and should perhaps be referred to it." To this Penhallow (4, p. 348) adds: "This species cannot be separated from the preceding on the characters given, and it is undoubtedly the same, though recognized here provisionally." If the two are identical the name for both will evidently be *P. Aldersoni*, since that name precedes the other in Knowlton's monograph.

Comparing *P. amethystinum* with living pines, we find that it has the ray tracheids of the Soft Pines (*Haploxyton*). It lacks tangential pitting in the summer wood, and thus resembles the Hard Pines (*Diploxyton*). If we ignore the tangential pitting or assume that this character was lost

in the petrification, this specimen comes near to *Pinus Lambertiana* or *P. flexilis*, both of which are Cordilleran species, the latter being common in the Park below 7000 feet. Both of these, however, have numerous and distinct tangential pits in the summer wood, and the preservation is so perfect in our specimen that it is hardly possible that details have been lost. *Pinus albicaulis* is said to have tangential pits on wood cells "not very numerous, rather small and flat, chiefly on outermost walls" (Penhallow, 4, p. 317). It would be best therefore to relate *Pityoxylon amethystinum* of the flanks of the Miocene volcano most closely to *Pinus albicaulis* which is now common above 8500 feet on the remnants of the same volcano. At present, however, this must remain a mere suggestion.

This paper is presented with the consent of Superintendent H. M. Albright (now Director of the National Park Service), to whom also I am indebted for the opportunity to obtain and to study this specimen. I am also indebted to the late Dr. F. H. Knowlton for advice and for the first section cut. The specimen and slides will be deposited in The Yellowstone Park Museum at Mammoth Hot Springs, Y. N. P.

GRINNELL COLLEGE,
GRINNELL, IOWA

LITERATURE CITED

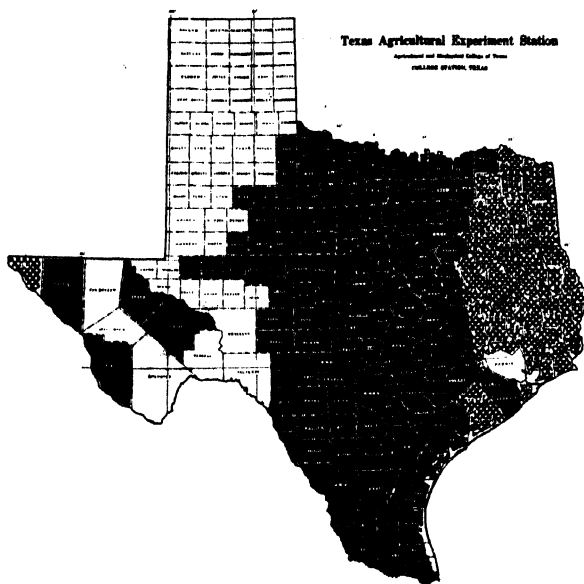
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RECENT STUDIES ON PHYMATOTRICHUM ROOT-ROT¹

J. J. TAUBENHAUS AND WALTER N. EZEKIEL

(Received for publication February 9, 1930)

Root-rot, caused by *Phymatotrichum omnivorum* (Shear) Duggar, occurs in Texas, parts of Arizona, Oklahoma, New Mexico, and Southern California, and recently it has been reported by Young (22) from Arkansas. Root-rot is also known to occur in parts of Mexico.



TEXT FIG. 1. Distribution of root-rot in Texas, December, 1929. Black areas indicate counties where root-rot causes considerable damage; shaded areas, counties where root-rot is known to be present but usually causes little loss; white areas, counties where root-rot has not been found or where no records available.

In 1923 (20) the disease was recorded in 67 counties of Texas. A recent compilation shows root-rot in 196 counties (text fig. 1). It is quite probable that as the studies continue, it will be found that the disease has even a wider distribution. Root-rot has not yet been found in the Plains country around Lubbock, nor in the extreme north of the Texas Panhandle. The absence of the disease from the extreme northern part of the state may be due to the severe winter weather, which may kill out roots of susceptible plants as well as the root-rot fungus itself.

¹ Published with the approval of the Director of the Texas Agricultural Experiment Station as Contribution no. 87, Technical Series, of the Station.

HOST PLANTS

Taubenhaus, Dana, and Wolff (17) have reported on the host range of root-rot. The disease was found to attack at least 274 species of cultivated plants, including important field crops, vegetables, fruit trees, berries, and ornamentals. Root-rot was found also on some 244 species of plants not ordinarily cultivated. These non-cultivated, and in many cases native, hosts are of importance from the standpoint of control, since perennial species may harbor the fungus for long periods of time. So far as is known, members of the grass family are apparently immune.

ECONOMIC IMPORTANCE OF ROOT-ROT

In estimating losses from root-rot on cotton, we are concerned less with the plant than with the seed and lint. A percentage count of infected or dead plants does not express the loss in yield, particularly in the case of plants that succumb late in the season. Infected plants may mature some bolls; however, as already indicated (20), the lint from such plants is of quality inferior to that of lint from normal plants. With crops such as alfalfa, losses from root-rot may be estimated fairly accurately by the curtailment in the life of the stand, the reduction in tonnage, and the lower quality of hay produced.

The writers estimate annual losses from root-rot at about 15 percent of the cotton crop of Texas. Damage from root-rot to legumes such as sweet clover, soybeans, and alfalfa, is highly important in that it makes difficult the growing of these crops in regions where the disease is prevalent. Root-rot is of enormous economic importance because it affects field and truck crops, orchard and nursery plantings, and even the ornamental plants, shrubs, and shade trees of towns and cities. We estimate the average aggregate annual loss from root-rot in Texas as around \$100,000,000.

SYMPTOMS OF ROOT-ROT

The symptoms of root-rot are more or less similar with all host plants. The first result noticed is a sudden wilting and drooping of affected plants. In early stages of infection, before complete wilting has occurred, affected plants may take on a slight yellow to a yellowish bronze tinge; and the top leaves droop and wilt even though the lower leaves are still turgid. With herbaceous plants, such as cotton or alfalfa, the foliage may wilt completely within ten to twelve hours after this first slight wilting. Such plants may revive at night to wilt permanently the following day. With shrubs and trees, the leaves yellow and gradually shed, perhaps during two seasons, before the final wilting.

Infected plants do not always die. Some plants recover by sending out new lateral roots above the infected part of the tap-root. Such plants may survive for a short time only, or may persist for months. Alfalfa plants appear to recover, by the development of superficial roots, following

irrigation periods. On the other hand, with cotton plants grown without irrigation, recovery appears to be more frequent during dry weather. Either of these extreme soil conditions evidently is unfavorable to the fungus.

Direct injury from root-rot is confined to the root systems and to the particular portions of the roots invaded. The root is first invested with the characteristic strands of the fungus, which are at first whitish but in a few days become yellowish to buff colored. Sharply depressed, dark brown lesions are produced at the points invaded. These lesions enlarge and merge, usually involving most of the surface of the tap-root and girdling it before the plant wilts, apparently from lack of water. The cortical tissues decay and soften before there is much injury to the central woody cylinder. Gradually, the disease involves more and more of the root system, which decays and is reduced finally to a soft, friable condition. There appears to be no general systemic injury to the roots, the uninvaded areas remaining alive often for months or years (depending on the host plant) after the top of the plant has been killed. Thus after the top of the plant has wilted and died and the tap-root has rotted and disintegrated into the soil, there may remain, at the periphery and below the area of infection, numerous ends of the roots, still alive and still harboring the fungus. Eventually the disease extends to the ends of the roots. In digging out fourteen two-year-old alfalfa plants at San Angelo, we found that with four plants, the fungus had followed the roots to a depth of about three feet; with six plants, infection had penetrated five feet; with the other four plants, the roots and the fungus had reached a depth of eight feet. In excavating several jujube trees in the vicinity of College Station, infection was found to follow these roots to a depth of eight feet in the ground.

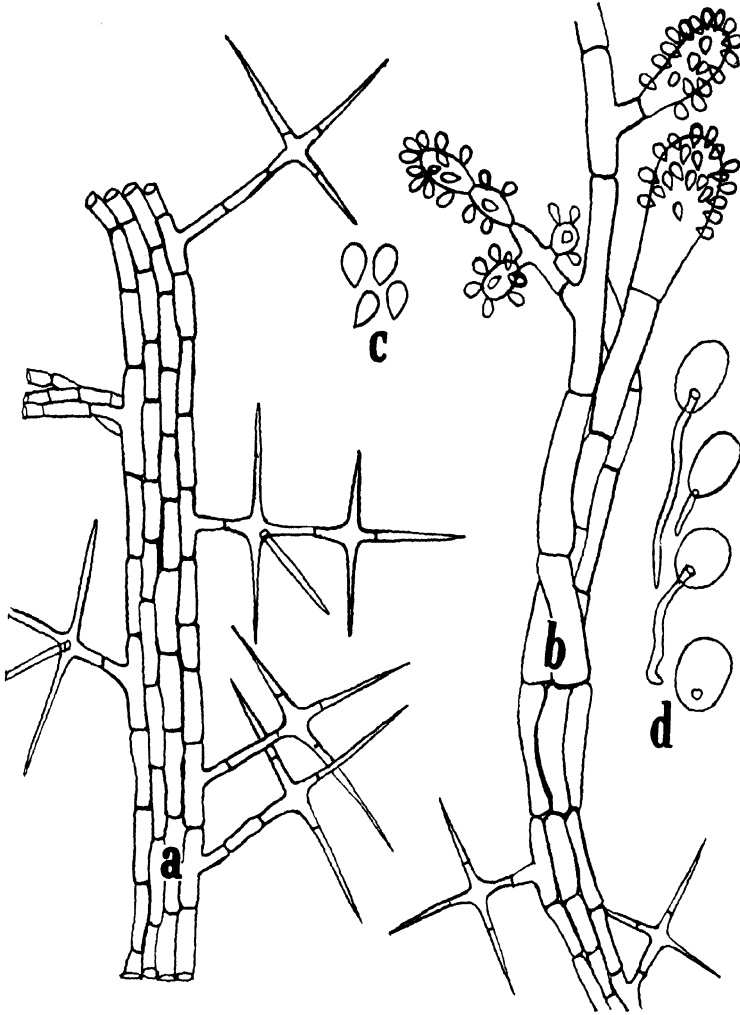
PHYMATOTRICHUM OMNIVORUM, THE FUNGUS WHICH CAUSES ROOT-ROT

The fungus, *Phymatotrichum omnivorum* (Shear) Duggar, develops profusely over infected roots, is readily isolated in pure culture, and successful inoculations from pure cultures have been accomplished (5, 18, 20). In recent work with the root-rot disease, a rapid field method for artificially inoculating plants on a large scale has been developed. The method (16) consists of inserting the tap-root or lateral roots of a freshly-infected plant close to the roots of the healthy plant, and watering copiously. With this method, high percentages of infection have been obtained in field inoculations with cotton and with alfalfa roots. Success has also been obtained in cross-inoculations on cotton and alfalfa with root inoculum from various hosts such as cotton, sweet clover, soybeans, carrots, beets, apples, pears, jujubes, grapes, and figs.

The Ozonium, or Vegetative Stage

The root-rot fungus was described originally (9, 13) from the sterile Ozonium stage. The Ozonium vegetative strands are invariably present on

the roots of infected plants. The yellow to buff-colored strands are composed of parallel hyphae which anastomose frequently. The individual hyphae resemble those of *Rhizoctonia* in the mode of branching and septation.



TEXT FIG. 2. Diagrammatic drawings of stages of *Phymatotrichum omnivorum*, the fungus that causes root-rot: *a*, an individual Ozonium strand with the accompanying typical acicular branches; *b*, morphological connection of conidia-bearing cells of *Phymatotrichum* above ground with the underground Ozonium strands beneath, as observed under the microscope; *c*, individual conidia of *Phymatotrichum omnivorum*; *d*, individual conidia of *Phymatotrichum omnivorum* showing different stages of germination. (*c* and *d* much enlarged.)

Surrounding these strands is the characteristic fuzzy coating, consisting of short upright hyphae, with typical acicular branches at the tips (text fig. 2, *a*).

The Phymatottrichum, or Conidial Stage

The conidial stage of the root-rot fungus was first described by Thornber (21), and was described and named as *Phymatottrichum omnivorum* by Duggar (2). This stage appears as spore-mats on the surface of the ground, usually around plants which have died from root-rot. Spore-mats are formed during moist weather in the spring, summer, or fall months.

A young spore-mat appears on the surface of the ground, first as a small fluffy, whitish mycelial growth. This increases rapidly to an elevated, flattened, cushion-like mass from 2 to 18 inches in diameter and usually less than one-fourth inch in thickness (Pl. XXXIII, *a*), consisting of *Rhizoctonia*-like hyphae with greatly inflated tips around which the minute conidia are borne. Eventually, the entire exposed surface of the spore-mat turns into a mass of buff-colored conidia, which gradually blow away. The spores themselves are hyaline, globose to ovate, and 4.5 to 7 microns in diameter (Pl. XXXIII, *c*).

The outer periphery of the mat extends in a zonate manner, the central zones being older and of a darker color (Pl. XXXIII, *b*). When sudden hot dry weather follows a wet period, the outer surface of the spore-mat may dry and retain the conidia. The writers have found such spore-mats to persist for several weeks. Occasionally, old spore-mats were observed to enlarge again during a return of rainy weather.

More than fifty spore-mats have been excavated and studied in the laboratory.² These mats were dissected carefully with needles and studied microscopically. It was possible to demonstrate actual continuity of hyphal growth from the subterranean strands, with characteristic acicular branching, to the conidia-bearing mycelium of the spore-mats above ground (text fig. 2, *b*). Together with the fact that the *Phymatottrichum* stage was found in pure culture (20), this leaves no doubt that the vegetative *Ozonium* stage and the *Phymatottrichum* conidial stage are actually stages of the same fungus. We found, also, that many spore-mats developing apparently away from infected plants were actually located over the still living, infected roots of cotton, alfalfa, or susceptible weeds.

In 1928, Lusk studied the spore-mats that appeared in an infected alfalfa field at Iowa Park Substation. Within an area of about one half acre, there was a first crop of 80 spore-mats, and a second of 70 spore-mats. A third crop of 18 spore-mats appeared in the field in the spring of 1929. Each of these three crops was carefully charted, and it was found that the spore-mats appeared to develop quite without regard to the location of mats of the previous crops.

We have obtained only low percentages of germination (text fig. 2, *d*) with the *Phymatottrichum* spores, and the germ tubes produced soon disintegrated. There is no evidence that such spores can cause infection.

² A large part of these studies on spore-mats was done by J. P. Lusk at the Iowa Park Substation.

Sclerotial, or Resting Stage

King and Loomis (6) first reported the discovery of true sclerotia in pure cultures in the laboratory of the United States Field Station at Sacaton Arizona. In the spring of 1929, Neal (8) found similar sclerotia in cotton fields at Greenville and at San Antonio, Texas.

Work during 1929 by the writers and their associates (18) has confirmed both observations. Large numbers of these sclerotia were found during the spring and summer of 1929, in excavations in infected cotton fields at the Blackland Substation at Temple, and at Rockwall and at Lancaster. Sclerotia were found also in fields of garden beets, sugar beets, and sweet potatoes in the vicinity of Wichita Falls and Fort Worth, Texas. Dana found sclerotia abundant in a carrot field (1), and sclerotia were found near the roots of infected fig trees by H. E. Rea at Temple, and by W. J. Bach at the Weslaco Substation. Sclerotia are also produced readily in the laboratory (6, 18). The individual sclerotia are fusoid to ovoid, with a dark buff-colored outer layer and lighter colored interior of pseudo-parenchymatous tissue (Pl. XXXIII, *f* and *g*). They are produced singly, as enlargements along the strands, in chains or in clusters.

Inoculation of Normal Cotton Plants with Sclerotia

Normal cotton plants were inoculated with sclerotia produced under natural conditions, and with a pure culture isolated from a sclerotium. Successful inoculations were obtained with both types of inoculum, while the check plants remained normal (18). This experiment proved that growth from sclerotia is able to infect normal cotton plants and produce typical root-rot. Sclerotia of *P. omnivorum* probably play an important rôle in the overwintering of the organism. Considering the ease with which sclerotia may be produced in the laboratory, they may serve also as a convenient source of inoculum in experiments.

Dormant Strands

Early in the season of 1929, cotton plants were found dying in fields which were in clean culture fallow during 1928, but which were in cotton attacked by root-rot in 1927. Excavations were made at Rockwall, Lancaster, and Itasca; and the observations suggested that infection of the 1929 plants was not only from sclerotia, but probably also from dark, nearly smooth strands which apparently had overwintered (Pl. XXXIII, *j*). These strands produced short tufts of typical hyphal growth when placed on moist soil or filter paper. Dana (1) has reported successful inoculation of one cotton plant with such strands. The writers consider these strands as probably similar to sclerotia except that they are formed under some conditions which inhibit enlargement of the strands into shapes more typical of sclerotia.

A Possible Perfect Stage

Shear (14) found a *Hydnum* on a plant dying from root-rot, and believing it to be the perfect stage of the root-rot fungus, named it *Hydnum omnivorum*. The writers have found a *Hydnum* (Pl. XXXIII, c-e) on cotton plants previously killed by *Phymatotrichum* root-rot and on the surface of the ground nearby. Pure cultures isolated from the tissues have persistently failed to show any resemblance to *Ozonium* or *Phymatotrichum*.

ENVIRONMENTAL FACTORS WHICH AFFECT THE OCCURRENCE AND SEVERITY OF ROOT-ROT

Soil Moisture and Temperature

The influence of rainfall and temperature variations during the growing season on the occurrence and severity of root-rot has been studied (15). In general, the lowered soil moisture supply during droughty periods checks the spread of root-rot, even though temperature conditions are favorable. With the early fall rains, renewed spread then occurs. The greatest numbers of wilted plants are found when favorable high temperature and moisture conditions occur together. It is to be noted that we know little, as yet, about the moisture and temperature conditions necessary for winter spread. The disease continues to spread on the roots during the fall and winter months, although affected plants do not wilt at that time, and there is no way of detecting the presence of the disease from above ground (18).

Soil Reaction

In the field, one notices that root-rot does considerable damage in some regions but is of less importance in others. The distribution of root-rot in Texas was studied in a survey, and found to be correlated with differences in soil reaction (19). The disease was more abundant and also more destructive in fields with neutral or alkaline soils, of pH 6.5 to 8.0; while it was less frequent and of little importance in acid soils of pH 5.5 to 6.4. These results agreed with the laboratory determination of the hydrogen-ion relations of *P. omnivorum* in artificial media (19).

Further insight into the relation of soil reaction to the presence or absence of root-rot was furnished by an experiment started in 1928. Soil of seven different types was dug out to a depth of three feet, and replaced, with each soil layer in its original depth, in wooden boxes at College Station. These boxes were 12 feet long, 3 feet deep, and 2 feet wide, and were sunk level with the surface of the ground to maintain normal temperatures. Cotton was planted in a row down the middle of each box, and root-rot was introduced by repeated artificial inoculation (16) at only one end of the row in each box. The results are given in table 1. It will be noted that no spread, and the smallest percentage of infection, occurred in the Susquehanna fine sandy loam and the Tabor fine sandy loam, shallow phase, both acid soils. The amount of root-rot was greatest in the most alkaline soils.

TABLE I. *Relation of Soil Reaction to Spread of Root-Rot during the Season of 1928 along Rows of Cotton, Each Inoculated Repeatedly at One End Only*

| Soil Type | pH of the Soil (0-2 Feet Deep) | Spread of Root-rot from End of Box, Feet | Percentage of Plants Infected |
|---|--------------------------------|--|-------------------------------|
| Susquehanna fine sandy loam, shallow phase..... | 5.5 | 0 | 2 |
| Tabor fine sandy loam, shallow phase..... | 5.8 | 0 | 0 |
| Ochlockonee clay loam..... | 6.3 | 4 | 27 |
| Tabor fine sandy loam..... | 6.7 | 4 | 29 |
| Kirvin fine sandy loam..... | 7.1 | 5 $\frac{1}{2}$ | 30 |
| Caddo fine sandy loam..... | 7.6 | 7 $\frac{1}{2}$ | 53 |
| Houston black clay..... | 7.7 | 7 $\frac{1}{2}$ | 74 |

In other experiments (3), the incidence of root-rot, its spread, and the number of plants killed, were again found to be correlated with the acidity or alkalinity of the soil. These results have suggested the possibility of controlling root-rot by acidifying the soil, and preliminary tests of this nature are under way. Under experimental conditions, it has been possible to control root-rot by acidifying the soil in containers to a pH of 5.0 (3). Application of the method under field conditions is being further studied.

STUDIES ON THE AVENUE OF SPREAD DURING THE GROWING SEASON

Root-rot spreads from plants infected early in the season to adjoining plants in the same row or in neighboring rows. It may take a few days or several weeks for the disease to progress from plant to plant, depending on temperature and moisture conditions in the soil and the distance between plants.

In a typical root-rot spot, the interior consists mainly of dead plants, and those few which have escaped or recovered from the effects of the disease. Around this region are more recently infected plants, in different stages of wilting, which in turn are surrounded by plants which appear normal but whose roots are already invaded by the fungus. The peripheral border of recently-infected plants constitutes the active zone of a root-rot spot. As indicated elsewhere (16), roots of such plants are themselves infective when pulled up and placed next to the roots of healthy plants.

Taubenhaus and Killough (20) have suggested that spread of root-rot occurs as the fungus extends along the roots of infected plants to the roots of adjoining healthy plants. Peltier, King, and Samson (10), on the other hand, considered that spread of root-rot is independent of roots, but that instead the fungus advances in a "fairy ring" fashion, launching out and advancing in the soil in all directions without regard to the distribution of roots. We summarize below the results of some studies on this question of the avenue of spread from infected roots to roots of neighboring plants.

A number of the experiments were based on the hypothesis that if the fungus advances into the soil independently of roots, the soil in the active

zones of root-rot spots should contain the advancing hyphae and might be infective when used as inoculum.

Cubes of Soil as Inoculum

In one series of inoculations, cubes of soil were taken from next to recently-wilted plants in the active zones of root-rot spots in cotton fields near College Station, and used to inoculate healthy cotton plants. Inoculations were made by removing soil from beside the healthy plant, watering heavily, and filling the hole with the soil inoculum from which all larger roots were first removed. Checks for these experiments consisted of similar healthy plants which were inoculated with freshly-infected cotton roots. The results are given in table 2. Not one of the 2,367 plants inoculated with the presumably infective soil showed any injury from root-rot; while 180 of the 220 check plants, inoculated with infected roots, were attacked by the disease.

TABLE 2. *Inoculations During 1927 of Cotton Plants with Soil from the Actively Advancing Edges of Root-Rot Spots. (Inoculations, Including Sept. 2, of Plants in Experimental Field at College Station; Thereafter, of Potted Plants in the Greenhouse)*

| Source of Inoculum | Inoculum | Date of Inoculation | No. of Plants Inoculated | Percent Infection |
|--|---|---------------------|--------------------------|-------------------|
| J. Zemanek farm, west of Bryan, Texas | Soil. Wilson clay | Aug. 10 | 20 | 0 |
| | " | Sept. 2 | 500 | 0 |
| | " | Oct. 5 | 60 | 0 |
| Farm south of Millican, Texas | Soil. Wilson clay | Aug. 10 | 57 | 0 |
| W. E. Graham farm, near College Station, Texas | Check. Live, freshly-infected cotton roots. | Aug. 12 | 70 | 78 |
| | Check. Live, freshly-infected cotton roots. | Oct. 5 | 15 | 38 |
| | Soil. Crockett clay | Aug. 15 | 500 | 0 |
| Farm north of Bryan, Texas | Soil. Lufkin clay loam. | Aug. 20 | 350 | 0 |
| | " | Aug. 22 | 300 | 0 |
| Williams farm, near Benchley, Texas | Check. Live, freshly-infected cotton roots. | Aug. 13 | 125 | 92 |
| | Check. Live, freshly-infected cotton roots. | Dec. 15 | 15 | 27 |
| | Soil. Crockett clay loam. | Aug. 11 | 100 | 0 |
| | " | Aug. 12 | 300 | 0 |
| | " | Oct. 6 | 120 | 0 |
| | " | Dec. 15 | 60 | 0 |

Soil-sifting Experiments

Soil material of three different types, Lufkin fine sandy loam, Wilson clay loam, and Crockett clay loam, was secured from active zones of root-rot spots. The soil was dug out to a depth of 3 feet. Some of the soil of



TEXT FIG. 3. Rows of cotton grown in 1928 in containers filled with Lufkin fine sandy loam originally taken from active root-rot zones. *a*, sifted soil, no root-rot appeared; *b*, unsifted soil, 83 percent root-rot.

each type was sifted to remove all roots which could not pass through a one-fourth inch mesh sieve; the unsifted soil contained all the infected roots which were originally present. Soil was placed in tanks 20 feet long, 4 feet wide, and 3 feet deep. No effort was made to place the soil material in the containers at the particular depths from which it had been secured, but instead soil from the various depths was intermixed during the sifting and other handling of the unsifted soil as well as the sifted soil. One container was filled with unsifted soil as secured, and one with sifted soil, for each of the soil types. A row of cotton was planted down the middle of each container, in 1928 and again in 1929. Root-rot appeared in each container of unsifted soil. No root-rot appeared in any of the sifted soils during either year (table 3, also text fig. 3).

TABLE 3. *Results of Soil-sifting Experiments at College Station, with Soil Secured from the Active Edges of Root-Rot Spots*

| Soil | Treatment | 1928 Results | | | 1929 Results | | |
|------------------------|------------------|-------------------------------|------------------------|--------------------------------|-------------------------------|------------------------|--------------------------------|
| | | Number of Centers of Root-rot | Total Number of Plants | Number of Plants with Root-rot | Number of Centers of Root-rot | Total Number of Plants | Number of Plants with Root-rot |
| Lufkin fine sandy loam | Sifted | 0 | 65 | 0 | 0 | 59 | 0 |
| | Unsifted | 2 | 56 | 47 | 4 | 62 | 47 |
| Wilson clay loam | Sifted | 0 | 71 | 0 | 0 | 59 | 0 |
| | Unsifted | 2 | 73 | 38 | 2 | 62 | 4 * |
| Crockett clay loam | Sifted | 0 | 77 | 0 | 0 | 56 | 0 |
| | Unsifted | 2 | 84 | 68 | 6 | 59 | 59 |

* Part of the unsifted, Wilson clay loam soil was excavated in the spring of 1929 in a study of the infected roots in this container, and the resultant drying apparently inhibited spread during 1929.

We have already mentioned the discovery (6, 8, 18) of sclerotial bodies. Considering the size of sclerotia, it is probable that if they were present in the soils used in these experiments some should have passed through the sieve and caused infection. Since no infection occurred during the two years, we may assume that sclerotia were not present in the active portions of root-rot spots from which the soil material was secured, at that particular time; that too few sclerotia passed through the sieve to produce infection; or that on account of the handling, all sclerotia present had germinated before cotton was planted.

Excavation of Infected Roots

If the root-rot fungus spreads in the soil independently of any roots, it should be possible to detect the spread by careful dissection and visual

examination of the soil around isolated infected plants. In coöperation with H. E. Rea, the writers excavated 117 infected cotton plants early in the spring of 1929. Isolated cotton plants, apparently representing initial infections, were located; a trench 3 feet deep, 4 feet wide, and about $1\frac{1}{2}$ feet away from each infected plant was then dug. This deep trench made it possible for the excavator to work with ease. The soil around the infected plant was then carefully dissected away with stiff needles and ice picks. Each soil particle as removed was carefully examined under the hand lens for the presence of root-rot hyphae, and all roots and rootlets were examined similarly. Doubtful material was studied under the microscope. After the soil was removed from the wilted plant, excavation and further dissecting of the soil was continued to the right and left to adjoining, apparently normal, plants to establish if possible how the fungus advances. We found no evidence of infection resulting from spread through the soil independently of roots, while in every excavation the fungus was found traveling along the lateral roots, and even along the finest of rootlets, and spreading from plant to plant apparently as the result of complete or proximate root contact.

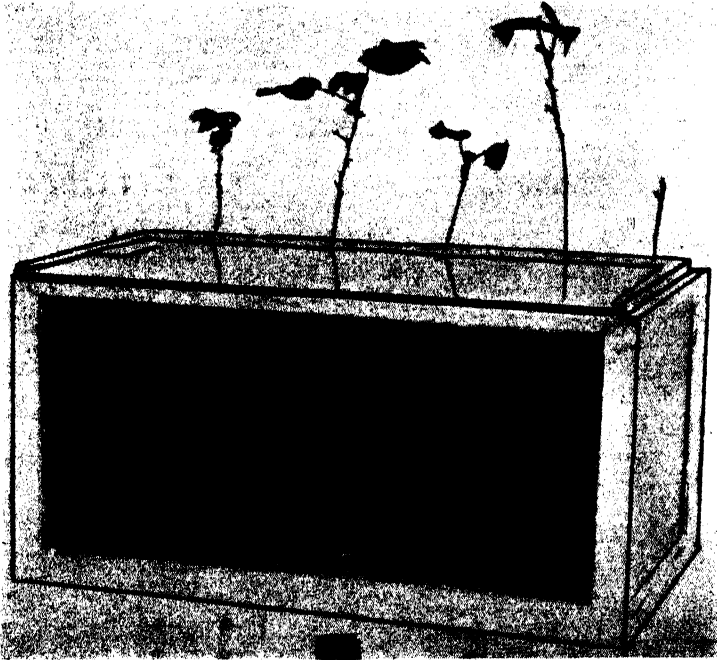
In excavating near the centers of well advanced root-rot spots, back of the region in which spread occurs, occasional isolated strands were found which appeared to be advancing in the soil independently of roots. In most of these cases, the strands were found to follow openings made by insects or earthworms. In other instances, fungus strands found in the soil were clinging to remnants of rootlets which had almost completely disappeared from decay. The finest rootlets offer an avenue of spread for the fungus, and may decay, leaving the accompanying fungus strands alone in the soil.

LABORATORY STUDIES ON SPREAD IN SOIL IN CONTAINERS

Further information as to spread of the root-rot fungus in the soil has been obtained from studies, commencing in November, 1928, on the growth of strands in soil in glass containers (text fig. 4). Oblong or vertical containers 8 inches wide, 8 inches deep, and 2 to 3 feet long were filled with moist Norfolk fine sand. Each box was inoculated at one end with a number of pieces of live, freshly-infected cotton roots, which were thrust into the soil. In all the containers there was a rapid development of mycelium of *Phymatotrichum* on the exposed ends of the inoculum, over the adjoining soil surface, and between the soil and the glass walls of the containers (text fig. 4).

It was of considerable interest to ascertain whether the extensive growth observed was confined to the upper surface of the soil and to the soil-glass surfaces, or whether it occurred also through the soil itself. The soil from a number of containers was carefully dissected, with needles, into individual particles, while it was examined with the hand lens and when necessary with the microscope. It was found that growth along the sides of the containers

was mainly along the glass walls. However, *Phymatotrichum* growth was not confined exclusively to these surfaces. At a few points, in the large amount of soil examined, there was actual penetration of the soil by loose, whitish wefts of *Phymatotrichum* mycelium, to a distance of 1 to 2 cm. only.



TEXT FIG. 4. *Phymatotrichum omnivorum* strands growing out from infected cotton root inoculum and spreading along the walls of glass containers of moist soil.

The mycelial growth that permeated the soil in these isolated points consisted of very loose, whitish aggregations of hyphae which appeared to grow mostly in crevices in the soil.

In later experiments, in which moist Houston black clay soil was used in these containers, heavy strand growth occurred along the glass surfaces as before, but in addition, the fungus was found to travel a distance of at least 5 cm. through the soil under these conditions. Inoculum was placed in the center of some containers as they were filled, and growth extended through the soil to the glass. In another container filled with moist Bell clay, the fungus traveled at least 10 cm. to the glass wall.

These studies show the possibility of spread of the fungus directly through the soil, at least in laboratory experiments with loose soil, although we have not yet found such spread in the field. Further laboratory studies are now in progress to determine the conditions affecting growth through the soil, and the maximum extent of growth possible under various conditions.

MODES OF OVERWINTERING

Taubenhaus and Killough (20) showed that root-rot may overwinter on live, infected roots. Survival of root-rot on infected roots was demonstrated also by the soil-sifting experiment described above (table 3). Roots of plants infected in fall may pass through the winter without being entirely rotted away. From these, the fungus continues to spread, even during winter, to adjoining, previously uninvaded roots. A number of lines of experimental evidence have suggested that the fungus probably survives in an active condition chiefly on living roots rather than on decayed roots (18). In making isolations from infected roots, the fungus is readily recovered from the margin of the infected area where it adjoins the normal tissue; while cultures have not been secured from the decayed parts of the roots (18). In inoculation experiments, we have been able to secure nearly a hundred percent of infection when using living though infected roots as inoculum; while no infections have been secured with infected but decayed roots.

To determine the extent to which cotton roots, for instance, actually winter over, numerous studies of roots have been made at intervals during the winter and spring months. Cotton roots were found to overwinter in all parts of Texas where cotton is grown. Even where the soil was disturbed by plowing, 50 to 80 percent of cotton roots from the previous season were still alive when the new crop was planted. As the season advanced, the old roots gradually died out, although a few were still alive in September.

The root-rot fungus overwinters not only on infected roots of cotton, but also of many other biennial and perennial plants. It occurs on newly-cleared land as a result of the persistence of infected, living, roots of native trees and shrubs (17). In rotations, root-rot survives not only on cotton roots but also on the roots of many susceptible weeds which frequently thrive on stubble land.

We have mentioned the recent discovery that the root-rot fungus produces resting bodies or sclerotia, which are apparently an important additional means of overwintering. Sclerotia, and dormant strands described above, have been found in fields from which infected roots were eliminated by clean fallow and were apparently the source of infection in the fields. Growth from overwintered strands was observed to spread and envelop an occasional dead root in the soil. It may be noted that Ratliffe (11) observed growth on such old roots, and concluded that the fungus had maintained itself saprophytically on these dead roots for a period of three years.

Extensive studies are now under way to determine the time and conditions under which sclerotia are formed, their importance in the life cycle of the root-rot fungus, and their longevity. We have observed that sclerotia are apparently parasitized by other microorganisms, and the longevity of sclerotia may be influenced by the presence of such parasites as well as by environmental conditions.

As yet, we do not know whether the fungus can overwinter in the conidial, *Phymatotrichum* spore stage, as *Hydnum* spores, or as some other possible spore stage.

PRESENT STATUS OF CONTROL

Present recommendations for the control of root-rot are necessarily of a temporary nature, since no completely satisfactory methods have as yet been established.

Whether the root-rot fungus overwinters on live roots, or on dead roots as Ratliffe (11) believes, as sclerotia or in some spore form, or as vegetative mycelium in the soil, control will be obtained if, by rotation and clean culture, the soil can be freed of all the means which enable the root-rot fungus to survive the winter months. The time required to accomplish complete eradication of all means of carry-over will have to be based on knowledge of the longevity in the ground of these possible agencies. Crop rotations, coupled with intensive clean-cultural practices, have already yielded encouraging results in reducing losses from root-rot. Reynolds and Killough (12) found that a four-year rotation reduced root-rot from 39.7 percent to 4.8 percent. McNamara (7) controlled root-rot by clean fallow for two years. In some unpublished results, H. E. Rea has found that rigid clean-cultural practices resulted in appreciable reductions in root-rot.

Development of varieties of plants resistant to root-rot would furnish an excellent solution of the problem. Many cotton varieties and selections have been tested, as yet without success. Attempts are also being made to find or develop resistant strains of alfalfa, grapes, citrus, and ornamentals. In the selection of apparently resistant plants, individual sound plants are not uncommonly found in root-rot spots, side by side with infected or dead plants. In digging out such "normal" plants, many have been found with signs of early infection followed by recovery, or with the tap-roots and lateral roots in various stages of active infection. Only rarely have plants been found with perfectly sound root systems although there were abundant possibilities for infection. We have noticed this condition particularly with cotton, and suggest that it might be well to observe the actual conditions of roots in attempts to select resistant plants in the field.

Several attempts to control root-rot by soil disinfectants have been reported (4, 5, 20). We have recently utilized a laboratory method for testing the efficiency of soil disinfectants against root-rot. This method consists of mixing the disinfectant into soil in definite proportions, placing the soil in glass containers along with inoculum, and observing growth through the soil and along the sides of the containers. Quantitative comparisons have been made of various disinfectants to determine the concentrations necessary to inhibit growth of root-rot strands in these containers. Results of these laboratory tests may not be directly applicable to field conditions, since in the field the question of penetration of the disinfectants

must be considered. Using rates of application based on the laboratory results, further tests are now under way with various materials in field plats.

It is not expected that disinfectants will be cheap enough to warrant their use over entire fields. Certain soil disinfectants may, however, prove useful in the control of root-rot on valuable shade and nursery trees; in the eradication of isolated spots in fields; and perhaps in use as barriers to prevent the spread of the disease from field to field.

In soils that are not highly calcareous, control of root-rot may eventually be possible by the application of sulfur to acidify the soil (3). Work on this phase is still in the experimental stage.

SUMMARY

Root-rot attacks a large proportion of cultivated and non-cultivated plants. Monocotyledons, including the grass and grain crops, have as yet appeared immune. The writers estimate the aggregate annual loss from root-rot in Texas as about one hundred million dollars.

The disease is characterized by sudden wilting of the foliage, followed by immediate or delayed death of the plant, or less frequently, by recovery. The roots are attacked and decayed by the fungus. Infection on alfalfa and jujube roots has been traced down to a depth of eight feet.

Root-rot is caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar. Cross-inoculations between various hosts have been successful. The conidial, *Phymatotrichum* stage is found on the surface of the ground above infected roots, and has been proved microscopically to connect with the typical vegetative or *Ozonium* strands which are found on the roots. The conidia germinate sparingly, and are not known to cause infection. Sclerotia and dormant strands have been found in the soil and cultured to prove their connection with the fungus. Sclerotia from the field, and a pure culture isolated from a sclerotium, have been used in successful inoculations of normal cotton plants in the laboratory.

Spread of root-rot during the growing season is favored by high temperatures and high moisture supply in the soil. Root-rot continues to spread on the roots during winter, although there is no evidence of wilting above-ground. The reaction of the soil appears important in determining the occurrence and severity of root-rot. The disease is favored by alkaline soils and inhibited in acid soils. This was proved by inoculation experiments as well as field results.

Studies are reported on the avenues of spread during the growing season. Soil taken from active zones of root-rot spots and used to inoculate healthy plants did not transmit the disease. Root-rot did not attack plants grown in soil from root-rot spots but sifted to remove roots; while it did occur in check containers filled with unsifted soil. Excavation studies of cotton plants, and examination of the soil and roots, failed to reveal any spread from these points of primary infection to adjacent roots through the soil independently of roots, which appeared to be the avenues of spread.

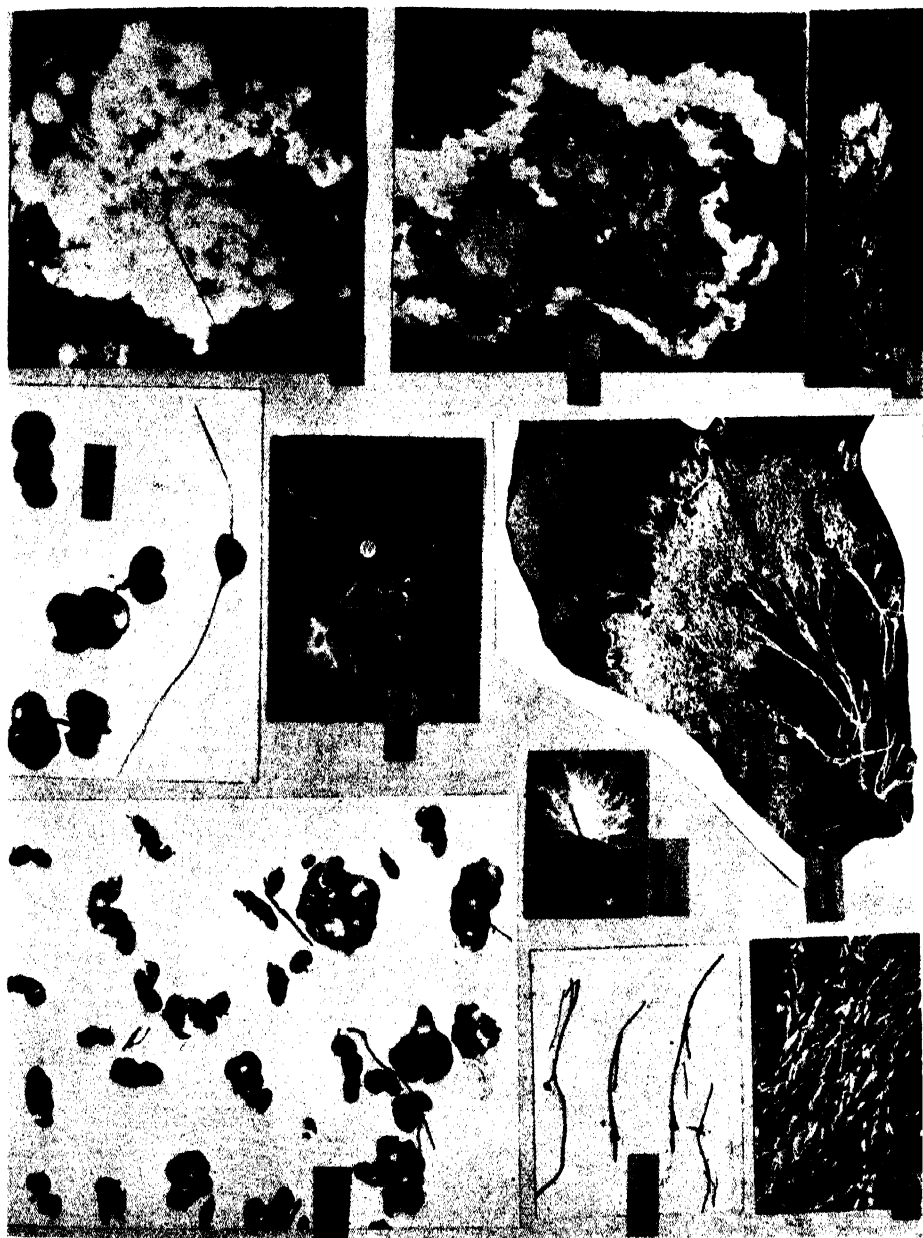
In laboratory experiments, the fungus developed profusely along the walls of glass containers, but penetrated only a few centimeters into the soil. In containers loosely filled with Bell clay soil, the fungus traveled at least 10 cm. through the loose soil to the glass wall.

The fungus has been shown to over-winter on live, infected roots, and as dormant strands and sclerotia. Clean culture and rotation with non-susceptible crops appear of value as control measures. Efforts to develop resistant varieties, and studies with soil disinfectants, are also discussed.

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EXPLANATION OF PLATE XXXIII

a and *b*, early and late stages in development of *Phymatotrichum* spore-mats.

c to *e*, *Hydnum*, possibly associated with *Phymatotrichum*. *c*, on cotton stem; *d*, on *Cocculus* leaf collected by B. F. Dana; *e*, enlarged view of the spiny sporophores.

f to *i*, sclerotial stage of *Phymatotrichum omnivorum*. *f*, enlarged view of sclerotia, showing a sclerotium borne as enlargement of main strand, and separated parts of chains of sclerotia; *g*, sclerotia separated from strands, showing chains and clumps of sclerotia; *h* and *i*, early and advanced stages of germination on agar.

j, dormant, apparently overwintered, strands of *Phymatotrichum omnivorum*.

A STUDY OF TWENTY-THREE OCTYL ALCOHOLS ON GROWTH OF *LUPINUS* SEEDLINGS

DAVID I. MACHT

(Received for publication February 10, 1930)

INTRODUCTORY

The present investigation has been rendered possible through the courtesy and kind coöperation of Dr. E. Emmett Reid, professor of organic chemistry, Johns Hopkins University. Under Professor Reid's guidance, Messrs. G. L. Dorrough, H. B. Glass, and G. B. Malone undertook the preparation and isolation in pure form of a series of isomeric octyl alcohols and the determination of their various chemical and physical constants. Theoretically, some fifty isomeric octyl alcohols are possible, and these chemists prepared twenty-three. Inasmuch as one of the most interesting subjects of pharmacology is the study of the relationship between chemical structure and physiological action of drugs and chemicals, the writer was very fortunate in obtaining the privilege of making a study of the various isomeric octyl alcohols thus secured, and grateful acknowledgement is hereby made to Professor Reid and his pupils for furnishing samples of these valuable compounds. Complete data on the subject, dealing with all kinds of zoöpharmacological and phytopharmacological experiments, will be published in a pharmacological journal. In the present paper, it was deemed desirable to report separately certain phytopharmacological studies which are of particular interest to the plant physiologist.

METHODS

A series of twenty-three isomeric primary, secondary, and tertiary pure octyl alcohols is included in the present study. Their names and structural formulae are exhibited in table 1. It will be noted that of the twenty-three octyl alcohols examined, four were octanols, eighteen were heptanols, and one was a hexanol. The table furthermore gives a primary, secondary, and tertiary classification of the various octyl alcohols.

In making a study of the various octyl alcohols, an attempt was first made to employ them in aqueous solution. Inasmuch as all of the octyl alcohols are but slightly soluble in water, a satisfactory, because more accurate, method was soon developed. The various octyl alcohols were first dissolved in 95 percent ethyl alcohol and such solutions in grain alcohol were employed as the starting-point for making dilutions in plant physiological solutions. In all the experiments, the solution for growing seedlings was made up of equal parts of distilled water and Shive's solution (1915).

TABLE I

[illegible]

To such a nutrient medium small quantities of alcoholic solutions of the octyls were added. Control solutions with ethyl alcohol alone were also made.

The phytopharmacological studies were made on seedlings of *Lupinus albus* in the same way in which these seedlings have been employed by the author and his collaborators in many other phytopharmacological investigations (1922, 1928). Seeds of *L. albus* are soaked overnight in tap water and then planted in finely ground, moist sphagnum moss. When the roots have attained a length varying from twenty to forty millimeters, they are carefully measured and the seedlings are placed in upright hard-glass tubes containing solutions of the various octyl alcohols, as well as the control solutions at 20° C. In the tables given below, the index of growth denotes the ratio of elongation of the roots in twenty-four hours, expressed as a percent of the growth of normal seedlings in control solutions.

RESULTS

Tables 2 and 3 indicate the results obtained. In table 2 various octyl alcohols are arranged in relation to the changing positions of the methyl group, while in table 3 they are arranged in relation to the changing positions of the hydroxyl groups. The small figures placed after and above the indices of growth indicate the number of series of experiments performed, of which the indices are the average. In each series of experiments, an average of ten seedlings was usually employed for each octyl alcohol. The most convenient concentration for comparative studies was found to be 1 : 10,000.

It will be noted, first, that the various isomeric octyl alcohols are not equally toxic for the growth of *Lupinus albus* seedlings. Further, it will be seen that, in general, the primary octyl alcohols are more toxic than the secondary and the secondary more toxic than the tertiary. It will also be noted that among the primary, secondary, and tertiary octyl alcohols quantitative differences also exist in regard to their respective toxicity for the plants. It may be well to state in this place that similar differences in all the respects mentioned above have been found by the author in zoöpharmacological studies.

EFFECT OF COMBINATIONS

The subject of synergism is one of the most important in modern pharmacology and refers to the effects obtained by combinations or mixtures of two or more drugs administered simultaneously or closely following each other. The pharmacologist has learned to recognize that when two or more drugs are administered to animals simultaneously, the result obtained may, in some cases, be an *additive* one, that is, a simple summation of the effects expected from the individual components in the quantities used. On the other hand, two or more drugs administered together may produce so-called *synergistic* effects, that is, physiological effects not explainable

TABLE 2. *Pharmacological Examination of Octyl Alcohol Toxicity for Living Seedlings of Lupinus albus as Compared with Normal Controls, Arranged in Relation to Changing Positions of Methyl Group*

| Number | Alcohol | Index of Growth in 1 : 10,000 | |
|--------|---------------------|------------------------------------|-----------|
| 1. | Octanol 1 | 44 ⁸ | PRIMARY |
| 2. | 2 methyl heptanol 1 | 56 ⁸ | |
| 3. | 3 methyl heptanol 1 | 52 ⁷ | |
| 4. | 4 methyl heptanol 1 | 61 ⁹ | |
| 5. | 5 methyl heptanol 1 | 57 ⁹ | |
| 6. | 6 methyl heptanol 1 | 55 ¹¹ | |
| 7. | Octanol 2 | 67 ⁹ | SECONDARY |
| 8. | Octanol 3 | 66 ⁷ | |
| 9. | Octanol 4 | 68 ⁶ | |
| 10. | 3 methyl heptanol 2 | 70 ⁸ (67 ⁷) | |
| 11. | 4 methyl heptanol 2 | 75 ⁸ | |
| 12. | 5 methyl heptanol 2 | 78 ⁷ (79 ⁸) | |
| 13. | 6 methyl heptanol 2 | 77 ⁸ (79 ⁹) | |
| 14. | 2 methyl heptanol 3 | 62 ⁹ | |
| 15. | 4 methyl heptanol 3 | 72 ⁸ | |
| 16. | 5 methyl heptanol 3 | 68 ⁷ (70 ⁸) | |
| 17. | 6 methyl heptanol 3 | 72 ⁸ (74 ⁹) | |
| 18. | 2 methyl heptanol 4 | 68 ⁹ | |
| 19. | 3 methyl heptanol 4 | 68 ⁸ | |
| 20. | 4 methyl heptanol 4 | 75 ⁹ | TERTIARY |
| 21. | 3 methyl heptanol 3 | 79 ⁹ | |
| 22. | 2 methyl heptanol 2 | 83 ⁹ | |
| 23. | 2 ethyl hexanol 1 | 54 ⁶ | PRIMARY |

TABLE 3. *Pharmacological Examination of Octyl Alcohol Toxicity for Living Seedlings of Lupinus albus as Compared with Normal Controls, Arranged in Relation to Changing Positions of Hydroxyl Group*

| Number | Alcohol | Index of Growth in 1 : 10,000 | |
|--------|---------------------|------------------------------------|----|
| 1. | Octanol 1 | 44 ⁸ | P. |
| 2. | Octanol 2 | 67 ⁹ | S. |
| 3. | Octanol 3 | 66 ⁷ | S. |
| 4. | Octanol 4 | 68 ⁶ | S. |
| 1. | 4 methyl heptanol 1 | 61 ⁹ | P. |
| 2. | 4 methyl heptanol 2 | 75 ⁸ | S. |
| 3. | 4 methyl heptanol 3 | 72 ⁸ | S. |
| 4. | 4 methyl heptanol 4 | 75 ⁹ | T. |
| 1. | 3 methyl heptanol 1 | 52 ⁷ | P. |
| 2. | 3 methyl heptanol 2 | 70 ⁸ (67 ⁷) | S. |
| 3. | 3 methyl heptanol 3 | 79 ⁹ | T. |
| 4. | 3 methyl heptanol 4 | 68 ⁸ | S. |
| 5. | 5 methyl heptanol 3 | 68 ⁷ (70 ⁸) | S. |
| 6. | 5 methyl heptanol 2 | 78 ⁷ (79 ⁸) | S. |
| 7. | 5 methyl heptanol 1 | 57 ⁹ | P. |
| 1. | 2 methyl heptanol 1 | 56 ⁸ | P. |
| 2. | 2 methyl heptanol 2 | 83 ⁹ | T. |
| 3. | 2 methyl heptanol 3 | 62 ⁹ | S. |
| 4. | 2 methyl heptanol 4 | 68 ⁹ | S. |
| 5. | 6 methyl heptanol 3 | 72 ⁸ (74 ⁹) | S. |
| 6. | 6 methyl heptanol 2 | 77 ⁸ (79 ⁹) | S. |
| 7. | 6 methyl heptanol 1 | 55 ¹¹ | P. |
| | 2 ethyl hexanol 1 | 54 ⁶ | P. |

by a simple summation of the component forces but exhibiting potentiation of one drug by another or an antagonistic action of one drug towards another. Such synergistic phenomena are of the greatest scientific and therapeutic importance to those dealing with drugs and are discussed at length by the author elsewhere (1929). The author has found that synergistic phenomena can be produced not only by combinations of two or more drugs of different chemical structure but also by combinations of chemical isomers, and even

by combinations of *stereoisomers*. It was therefore deemed of interest to examine various combinations of the octyl alcohols on the growth of *Lupinus albus* in connection with the present research. Table 4 illustrates some of the findings. It will be noted that certain combinations of the octyl alcohols produced synergistic effects, while others did not. Again, the synergism in case of certain combinations was manifested as a potentiation, indicated by a greater toxicity of the combinations than that of their individual components. In other cases, the synergism was of an antagonistic character and the combination produced a less toxic effect than would be expected by the simple arithmetical mean of the components.

TABLE 4. *Effect of Combinations of Octyl Alcohols on Growth of Lupinus Albus*

| Octyl Alcohols | Index of Growth in 1 : 10,000 | Calculated Arithmetical Mean |
|----------------------|----------------------------------|---------------------------------|
| I + II..... | 65 | 50 |
| III + IV..... | 76 | 57 |
| III + IX..... | 64 | 60 |
| III + XXII..... | 76 | 68 |
| III + IX + XXII..... | 56 | 68 |
| XX + XXI..... | 105 | 77 |
| XX + XXII..... | 92 | 79 |
| XXI + XXII..... | 56 | 81 |
| XX + XXI + XXII..... | 113 | 77 |
| I + VII..... | 52 | 56 |
| II + VII..... | 74 | 62 |
| I + X..... | 70 | 57 |
| I + XIV..... | 62 | 53 |
| X + XIV..... | 93 | 66 |
| I + VII + X..... | 57 | 60 |
| VII + X + XIV..... | 107 | 66 |
| VII + X + XX..... | 101 | 71 |

SUMMARY

The investigation described above brings out the following facts:

1. The twenty-three isomeric octyl alcohols were found to have different degrees of toxicity for *Lupinus albus* seedlings.
2. A definite difference in toxicity was found between the primary, secondary, and tertiary octyl alcohols, the primary being the most toxic and the tertiary being the least toxic.
3. Differences in toxicity between the individual members of each group were also noted.
4. Combinations of the various octyl alcohols exhibited synergistic effects.
5. The results of the investigation emphasize the importance of employing pure chemicals in pharmacological research.
6. An important conclusion to be drawn from the data given above is that in endeavoring to trace a relationship between chemical structure and

pharmacological effects no sweeping generalizations are permissible, but that every statement must be checked by actual experiment.

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GROWTH AND NITROGEN METABOLISM OF SQUASH SEED- LINGS III. WITH RESPECT TO HIGH AND LOW CARBOHYDRATE SYNTHESIS ¹

MARY E. REID

(Received for publication November 30, 1929)

INTRODUCTION

Fischer (6), Bornemann (3), Reinau (15), Lundegardh (8), Arthur (1), and others have studied the effects of added carbon dioxide upon vegetative growth and sexual reproduction. These investigators report increased size and dry weights of many types of plants as a result of enrichment of the atmosphere with carbon dioxide. More recently, Bolas and Henderson (2) have found that addition of carbon dioxide to the air results in a large increase in the dry weight of cucumber plants as compared to results obtained with plants grown in normal air. The increase is evident within two to three days after adding the carbon dioxide.

Reese (10) obtained beneficial effects by increasing the carbon dioxide content of the atmosphere in which seedlings and cuttings were growing. Leaves of *Aconitum Wilsoni* seedlings were three times larger than those of untreated plants. Chrysanthemum and dahlia cuttings rooted more quickly when grown in a carbon dioxide-enriched atmosphere.

In previous papers (11, 12) it has been shown that the ability of seedlings to utilize carbon dioxide depends on the amount of available nitrogen in relation to that of carbohydrates. Seedlings grown from seeds having large reserves of nitrogen in proportion to the non-nitrogenous materials benefited greatly from the utilization of carbon dioxide both when extra nitrogen was supplied and when it was not. The green weights (dry weights not determined) and sizes of all organs increased and that of the roots especially.

Seedlings grown from seeds having small reserves of nitrogen in proportion to the carbohydrates (wheat and low-protein corn) were not benefited by supplying carbon dioxide unless extra nitrogen was also given. The dry weights of the organs increased slightly but the sizes did not. In fact the leaves were somewhat stunted. With an abundance of nitrogen available in the substrate, the supplying of carbon dioxide was beneficial, especially in the later stages of seedling growth when the reserves of the seed were becoming depleted. A healthy condition and relatively large size of leaves could be produced on the one hand when the nitrogen supply was limited

¹ These investigations were conducted during 1928 in the Department of Physiological Chemistry of Yale University where the writer held a Sterling Research Fellowship.

by also limiting carbohydrate synthesis, and on the other by furnishing additional carbon (CO_2) in conjunction with the additional nitrogen. The supplying of additional nitrogen permitted the utilization of much more carbohydrate material and consequently resulted in the production of a much greater total leaf area than did the restricting of carbohydrate synthesis. It thus appears that the limitation in growth associated with carbohydrate synthesis in these experiments was due to a lack of balance between the available supply of carbohydrates and nitrogen.

Decomposition of Chlorophyll during Photosynthesis

Several investigators have observed changes, particularly a diminution of chlorophyll in the chloroplast-containing tissues of plants during periods of very active photosynthesis.

Sachs (16) suggested that the photosynthetic process itself results in degenerative changes in the chloroplasts. This suggestion, however, has not been accepted by most of the more recent investigators.

Schimper (18) stated that the protoplasmic substance of leucoplasts is more quickly and essentially changed or used up during its functional activity than is that of the chloroplasts.

Willstätter and Stoll (23) found no alteration in the chlorophyll content of leaves before and after a period of very active photosynthesis. It seems possible, however, that decomposition of some of the chlorophyll present in the plastids and the synthesis of a new supply might occur at approximately the same rate, even under conditions of rapid photosynthesis, provided an abundance of nitrogen is available and that there may be a capacity for its rapid utilization. Spoehr (19) did not express a view as to whether or not chlorophyll is destroyed during photosynthesis but states that it is doubtful if it is successively decomposed and reformed.

Miss Henrici (7) reports decreases in the chlorophyll content of grasses in Bechuanaland during drought periods and increases following rains. She also finds variations in chlorophyll content of leaves at different times of day and night. The chlorophyll content decreases from early morning to mid-day and increases again the next night. An understanding of the factors inducing these changes might be of considerable value in helping to account for variations in type of growth resulting from differences in light intensity and possibly in duration of illumination periods.

The wheat and low-protein corn seedlings previously mentioned (11, 12) grown without an external supply of nitrogen, some in atmospheres containing carbon dioxid, others in atmospheres lacking carbon dioxid, had definitely noticeable differences in the chlorophyll content of the leaves, those not permitted to synthesize carbohydrates being much greener and physiologically younger in appearance. Although a cytological study of these tissues has not been completed it seemed from a preliminary examination that there is more saffranin-stainable material in the chloroplasts which did not synthesize carbohydrates.

The leaves and foliaceous cotyledons of seedlings grown from much higher protein types of seeds and without additional nitrogen were somewhat greener in the intermediate stages of growth when permitted to synthesize carbohydrates than when the function was inhibited through lack of carbon dioxide. Apparently a very small supply of carbohydrates in proportion to nitrogen is unfavorable to the normal development of the chlorophyll.

The experiments with squash seedlings here to be described show the effects of synthesis of approximately just enough carbohydrates to allow the utilization of the reserve nitrogen in contrast to the effects of synthesis of an abundance of carbohydrates. Special attention has been paid to sizes and weights of different organs of the seedlings, color of cotyledons and leaves at different stages of growth, appearance of the chloroplasts, and allocation of nitrogen in the tissues.

Effect upon the Chloroplasts of Supplying Carbohydrates Artificially

Molliard and Matruchot (9) found that dextrose and levulose were rapidly utilized by the alga *Stichococcus bacillaris* and that these sugars caused the chloroplasts to become somewhat yellowed. Disaccharids such as cane sugar, maltose, and lactose were assimilated more slowly by the protoplasm. Under the latter conditions the green color of the cell was preserved.

Chodat (4) studied the growth of algae in pure cultures supplied with different types and different proportions of nitrogenous and carbohydrate compounds. He observed that the decomposition of chlorophyll together with other degenerative changes in the chloroplasts and an extensive production of fat in which the yellow pigments were dissolved occurred only under certain conditions of nutrition. His experiments in general show that it is the more rapid assimilation of sugar in relation to that of nitrogen which causes the loss of chlorophyll. The presence of a small amount of chlorophyll in some types of algae grown on a glucose medium appears to be primarily a degeneration phenomenon; in other forms, however, synthesis of chlorophyll as well as maintenance is affected since a weakness of color appears even at the beginning when the algae are growing vigorously.

In view of our present knowledge concerning the problems under consideration the following questions appear to be of major importance: (1) What are the effects of an abundance of carbohydrates supplied artificially or as a result of synthesis in conjunction with a limited supply of available nitrogen upon the metabolism of the pigment-plasma compounds of the chloroplasts? (2) Do such conditions produce a limitation in the synthesis of these compounds or is there difficulty of maintenance, or are synthesis and maintenance both affected?

Effect of Sugar Feeding on Blood Regeneration in Animals

Whipple and his associates have conducted extensive investigations on the effect of various foods on red blood cell and hemoglobin regeneration.

They reduced the hemoglobin and red cell content of dog's blood to a very low level by hemorrhages and then studied the multiplication of red cells and regeneration of hemoglobin after feeding different diets. They discovered that a considerable regeneration occurred during a starvation period and that more red cells and more hemoglobin were formed during such a fasting period than during a period of sugar feeding (22). The figures given below, which summarize the results of several of their experiments, are above the maintenance supply of hemoglobin which is required to keep the level uniform.

| | During Two Weeks | | | During Three Weeks | | |
|---|------------------|-------------------------|------------|--------------------|-------------------------|------------|
| | Pigment Volume | Total Hematocrit R.B.C. | Hemoglobin | Pigment Volume | Total Hematocrit R.B.C. | Hemoglobin |
| Nine expts. Fasting average . . . | 198 | 8.85 | 21 | 274 | 10.58 | 29 |
| Eight expts. Sugar average | 133 | 4.63 | 12 | 78 | 5.53 | 13 |

The investigators account for the above experimental results thus: When tissue protein is broken down the split products become available for the synthesis of hemoglobin. If carbohydrates are obtainable for the production of energy, however, the breakdown of tissue protein is reduced to a minimum and the necessary materials for the synthesis of hemoglobin are no longer set free. They state that they have no reason to suppose that the daily wastage of red cells is greater on sugar feeding than during fasting periods. They found in some (21) although not in all of their experiments that the total bile pigment output is increased by feeding carbohydrates exclusively. It has generally been supposed that bile pigments result from hemoglobin destruction but Whipple accepts the hypothesis that they may arise directly from surplus pigment-building materials (20).

The effect of carbohydrates on hemoglobin synthesis and possibly on maintenance as indicated by the work of Whipple and his associates appears to be somewhat similar to the effects of these compounds on the synthesis and maintenance of the plasma and chlorophyll content of chloroplasts. A number of physiologists have suggested the possibility of relationships between the plasma-pigment complex of the red corpuscle of the animal organism and the green one of the plant. If such relations do exist, it would be expected that there might be parallelisms in the nutritive conditions affecting the synthesis and maintenance of these substances.

METHODS

Hubbard squash seeds of uniform size were washed in distilled water, after which they were allowed to stand for an hour in a 0.2-percent solution

TABLE 1. *Seedlings Grown Without an External Source of Nitrogen in Atmospheres Having Different Concentrations of Carbon Dioxid.*
Twenty-eight Plants. Seeds Contained 328.0 mg. N. Sept. 13-Oct. 5, 1928

| Organs of Seedlings | Green Weights, gm. | Dry Weights, gm. | Percentage of Nitrogen in Dry Weight | Percentage of Nitrogen in Green Weight | Percentage of Moisture | Total Nitrogen, mg. | Total Leaf Area, sq. cm. | Mg. N. per sq. cm. |
|---------------------|--------------------|------------------|--------------------------------------|--|------------------------|---------------------|--------------------------|--------------------|
|---------------------|--------------------|------------------|--------------------------------------|--|------------------------|---------------------|--------------------------|--------------------|

Seedlings grown in an atmosphere containing approximately 1% CO₂

| | | | | | | | | |
|-------------------------------------|--------|--------|------|-------|------|-----|------|-------|
| Leaf I..... | 10.54 | 1.109 | 2.39 | 0.251 | 89.5 | 27 | 435 | 0.060 |
| Leaf II..... | 8.00 | 0.733 | 3.56 | 0.326 | 90.8 | 26 | 311 | 0.084 |
| Leaf III..... | 6.51 | 0.655 | 3.87 | 0.389 | 90.0 | 25 | 228 | 0.111 |
| Leaves IV and V..... | 3.25 | 0.322 | 5.03 | 0.498 | 90.1 | 16 | 93 | 0.174 |
| Total for leaves..... | 28.30 | 2.819 | | | | 94 | 1067 | |
| Stems, hypocotyls and petioles..... | 88.96 | 6.704 | 1.48 | 0.112 | 92.5 | 99 | | |
| Roots..... | 66.51 | 2.507 | 2.80 | 0.105 | 96.2 | 70 | | |
| Cotyledons..... | 39.46 | 2.693 | 1.63 | 0.111 | 93.2 | 44 | | |
| Total..... | 223.23 | 14.723 | | | | 307 | | |
| Loss of N..... | | | | | | -21 | | |

Seedlings grown in an atmosphere containing a very low concentration of CO₂

| | | | | | | | | |
|-------------------------------------|--------|-------|------|-------|------|-----|------|-------|
| Leaf I..... | 8.34 | 0.607 | 5.84 | 0.425 | 92.7 | 35 | 386 | 0.092 |
| Leaf II..... | 7.08 | 0.600 | 8.30 | 0.703 | 91.5 | 50 | 434 | 0.115 |
| Leaf III..... | 4.00 | 0.340 | 9.43 | 0.801 | 91.5 | 32 | 203 | 0.158 |
| Leaves IV and V..... | 0.86 | 0.079 | 9.43 | 0.866 | 90.8 | 7 | 47 | 0.159 |
| Total for leaves..... | 20.28 | 1.626 | | | | 124 | 1070 | |
| Stems, hypocotyls and petioles..... | 57.09 | 1.982 | 3.73 | 0.129 | 96.5 | 74 | | |
| Roots..... | 38.06 | 1.424 | 2.68 | 0.100 | 96.2 | 38 | | |
| Cotyledons..... | 44.35 | 2.112 | 3.13 | 0.149 | 95.2 | 66 | | |
| Total..... | 159.78 | 7.144 | | | | 302 | | |
| Loss of N..... | | | | | | -25 | | |

Seedlings grown in darkness

| | | | | | | | | |
|------------------------|--------|-------|------|-------|------|-----|--|--|
| Leaves..... | 1.84 | 0.097 | 8.75 | 0.461 | 94.7 | 8 | | |
| Stems, hypocotyls..... | 121.15 | 3.061 | 5.52 | 0.139 | 97.5 | 169 | | |
| Roots..... | 21.22 | 1.406 | 1.34 | 0.088 | 93.3 | 19 | | |
| Cotyledons..... | 17.42 | 1.252 | 8.45 | 0.607 | 92.8 | 106 | | |
| Total..... | 161.63 | 5.816 | | | | 302 | | |
| Loss of N..... | | | | | | -26 | | |

of Uspulun. They were then rinsed in redistilled water and placed in germinating dishes between layers of moist filter paper.

When the radicles were 1 to 2 cm. long, the seed coats and green membranes were carefully removed and the seedlings were planted in sterilized #3 quartz sand in seven-inch clay azalea pots which also had been sterilized. Some of the cultures were placed in the bell jar apparatus previously described (II) and designed for furnishing atmospheres containing different concentrations of carbon dioxide. The apparatus was placed in the greenhouse and was connected with the vacuum system of the laboratory. Suction was maintained on the atmosphere of the culture chambers sufficient to lift a column of water to a height of 7 cm.

TABLE 2. *Average Dimensions (cm.) of Different Organs of Seedlings of Experiment I. September 13–October 5, 1928*

| Organs of Seedlings | Light, High Carbon Dioxid | | Light, Low Carbon Dioxid | | Darkness | |
|---------------------|---------------------------|-------|--------------------------|-------|----------|-------|
| | Length | Width | Length | Width | Length | Width |
| Hypocotyl..... | 8.8 | | 8.1 | | 32.4 | |
| Stem..... | 8.7 | | 6.8 | | 1.0 | |
| Roots..... | 21.4 | | 18.5 | | 10.4 | |
| Cotyledons..... | 5.5 | | 5.3 | | 2.3 | 1.45 |
| Leaf I..... | 3.7 | 4.6 | 3.4 | 4.6 | 1.0 | 1.2 |
| Leaf II..... | 3.4 | 4.1 | 3.5 | 4.7 | | |
| Leaf III..... | 2.8 | 3.4 | 2.7 | 3.3 | | |
| Leaf IV..... | 1.9 | 2.2 | 1.3 | 1.4 | | |

The air entering the system of bell jars was drawn through a solution of sulfuric acid to remove any traces of ammonia which might be present. The air leaving the system was also drawn through sulfuric acid. The object in drawing the air from the respiration chambers through acid was to determine whether any nitrogen in basic form that could be absorbed by acid might be present.

In some of the previous experiments it had been impossible to establish a nitrogen balance, *i.e.*, less nitrogen was found in the seedlings than had been present in the seeds. It seemed that there might be three possible sources of the loss: (1) Nitrogen compounds might have been excreted by the roots and might have been assimilated and probably to some extent decomposed by micro-organisms. (2) The Kjeldahl method of analysis might have failed to detect a certain form or forms of the nitrogen compounds. (3) Gaseous nitrogen might have been respired.

The nutrient solution used in these experiments was prepared according to the following formula:

0.2% magnesium sulfate
0.2% monobasic potassium phosphate
0.1% dibasic potassium phosphate

0.2% calcium chlorid
0.15% calcium sulfate

TABLE 3. *Seedlings Grown Without an External Source of Nitrogen in Atmospheres Having Different Concentrations of Carbon Dioxid. Twenty-eight Plants. Seeds Contained 328 mg. N. October 10-31, 1928*

| Organs of Seedlings | Green Weights, gm. | Dry Weights, gm. | Percentage of Nitrogen in Dry Weight | Percentage of Nitrogen in Green Weight | Percentage of Moisture | Total Nitrogen, mg. | Total Leaf Area, sq. cm. | Mg. N. per sq. cm. |
|--|--------------------|------------------|--------------------------------------|--|------------------------|---------------------|--------------------------|--------------------|
| Seedlings grown in an atmosphere containing approximately 1% CO ₂ | | | | | | | | |
| Leaf I..... | 12.64 | 1.169 | 2.08 | 0.192 | 90.7 | 24 | 554 | 0.044 |
| Leaf II..... | 7.09 | 0.884 | 2.36 | 0.294 | 87.5 | 21 | 322 | 0.063 |
| Leaf III..... | 6.19 | 0.927 | 2.58 | 0.386 | 85.0 | 24 | 310 | 0.077 |
| Leaves IV and V..... | 5.13 | 0.738 | 3.63 | 0.522 | 85.6 | 27 | 199 | 0.134 |
| Total for leaves..... | 31.05 | 3.718 | | | | 96 | 1385 | |
| Stems + petioles..... | 60.68 | 4.712 | 1.48 | 0.115 | 92.2 | 70 | | |
| Hypocotyls..... | 33.67 | 2.833 | 1.20 | 0.101 | 91.6 | 34 | | |
| Roots..... | 71.01 | 3.021 | 2.20 | 0.093 | 95.7 | 66 | | |
| Cotyledons..... | 33.14 | 2.290 | 2.20 | 0.152 | 93.9 | 50 | | |
| Total..... | 229.55 | 16.574 | | | | 316 | | |
| Loss of N..... | | | | | | -12 | | |
| Seedlings grown in atmosphere containing approximately 0.02% CO ₂ | | | | | | | | |
| Leaf I..... | 8.55 | 0.683 | 4.61 | 0.368 | 92.0 | 31 | 442 | 0.071 |
| Leaf II..... | 7.19 | 0.630 | 6.50 | 0.569 | 91.2 | 41 | 398 | 0.103 |
| Leaf III..... | 5.72 | 0.546 | 7.98 | 0.761 | 90.5 | 44 | 398 | 0.109 |
| Leaves IV and V..... | 3.59 | 0.358 | 9.13 | 0.910 | 90.0 | 33 | 222 | 0.147 |
| Total for leaves..... | 25.05 | 2.217 | | | | 149 | 1460 | |
| Stems + petioles..... | 32.64 | 1.399 | 3.00 | 0.129 | 95.7 | 42 | | |
| Hypocotyls..... | 25.93 | 0.902 | 2.38 | 0.082 | 96.5 | 21 | | |
| Roots..... | 33.54 | 1.218 | 2.41 | 0.087 | 96.4 | 29 | | |
| Cotyledons..... | 39.39 | 2.212 | 3.54 | 0.199 | 94.4 | 78 | | |
| Total..... | 156.55 | 7.948 | | | | 319 | | |
| Loss of N..... | | | | | | -9 | | |

TABLE 4. *Seedlings Grown Without an External Supply of Nitrogen. Some in Light; Others in Darkness. Twenty-eight Plants. Seeds Contained 328 mg. N. October 10-31, 1928*

| Organs of Seedlings | Green Weights, gm. | Dry Weights, gm. | Percentage of Nitrogen in Dry Weight | Percentage of Nitrogen in Green Weight | Percentage of Moisture | Total Nitrogen, mg. | Total Leaf Area, sq. cm. | Mg. N. per sq. cm. |
|---|--------------------|------------------|--------------------------------------|--|------------------------|---------------------|--------------------------|--------------------|
| Seedlings grown in light in normal atmosphere | | | | | | | | |
| Leaf I..... | 15.38 | 1.771 | 2.19 | 0.252 | 88.5 | 39 | 590 | 0.066 |
| Leaf II..... | 9.06 | 1.225 | 2.92 | 0.395 | 86.4 | 36 | 352 | 0.101 |
| Leaf III..... | 4.83 | 0.672 | 3.80 | 0.528 | 86.1 | 26 | 153 | 0.167 |
| Leaf IV..... | 1.18 | 0.145 | 5.19 | 0.637 | 87.7 | 7 | 26 | 0.281 |
| Total for leaves..... | 30.45 | 3.813 | | | | 108 | 1121 | |
| Stems + petioles..... | 35.25 | 3.144 | 1.69 | 0.151 | 91.1 | 53 | | |
| Hypocotyls..... | 25.86 | 3.248 | 1.02 | 0.128 | 87.4 | 33 | | |
| Roots..... | 81.74 | 3.483 | 1.91 | 0.081 | 95.7 | 66 | | |
| Cotyledons..... | 38.26 | 3.004 | 1.47 | 0.115 | 92.1 | 44 | | |
| Total..... | 211.56 | 16.692 | | | | 304 | | |
| Loss of N..... | | | | | | -24 | | |
| Seedlings grown in darkness | | | | | | | | |
| Leaves, petioles and stems..... | 6.93 | 0.194 | 7.56 | 0.212 | 97.2 | 15 | | |
| Cotyledonary stems..... | 13.09 | 0.333 | 6.22 | 0.158 | 97.4 | 21 | | |
| Hypocotyls..... | 116.41 | 2.364 | 5.40 | 0.109 | 97.9 | 128 | | |
| Roots..... | 19.17 | 0.646 | 2.60 | 0.088 | 96.6 | 17 | | |
| Cotyledons..... | 21.54 | 1.216 | 8.36 | 0.472 | 94.3 | 102 | | |
| Total..... | 177.14 | 4.753 | | | | 283 | | |
| Loss of N..... | | | | | | -45 | | |

Fresh solutions were prepared for each application, using ammonia-free water. Seeds of uniform size and from the same lot were used in each of the two experiments herein described. The amount of nitrogen contained in each seed averaged 11.7 mg. In order to make comparisons readily with the results of the experiments previously described (13, 14) with seedlings grown at different seasons of the year, the calculations given in tables 1, 3, 4, and 5 were based on the amounts of nitrogen (324-328 mg.)

TABLE 5. *Average Dimensions (cm.) of Different Organs of Seedlings of Experiment 2. October 10-31, 1928*

| Organs of Seedlings | Light, High Carbon Dioxid | | Light, Low Carbon Dioxid | | Light, Normal Atmosphere | | Darkness | |
|---------------------|---------------------------|-------|--------------------------|-------|--------------------------|-------|----------|-------|
| | Length | Width | Length | Width | Length | Width | Length | Width |
| Hypocotyl..... | 7.2 | | 6.3 | | 5.3 | | 31.7 | |
| Stem..... | 9.4 | | 7.7 | | 4.3 | | 0.7 | |
| Roots..... | 26.6 | | 17.7 | | 27.5 | | 12.6 | |
| Cotyledons.... | 4.4 | 2.6 | 4.4 | 2.7 | 5.1 | 3.4 | 2.3 | 1.6 |
| Leaf I..... | 3.9 | 5.2 | 3.9 | 4.7 | 4.4 | 5.8 | 0.8 | 0.83 |
| Leaf II..... | 3.4 | 4.1 | 3.9 | 4.5 | 3.4 | 4.4 | | |
| Leaf III..... | 3.4 | 4.0 | 3.9 | 4.5 | 2.5 | 2.9 | | |
| Leaf IV..... | 2.6 | 3.1 | 2.8 | 3.1 | 1.1 | 1.2 | | |
| Leaf V..... | 0.97 | 1.1 | 0.71 | 0.87 | | | | |

TABLE 6. *Distribution of Total Nitrogen in Different Organs of Plants, Some Grown in Light in Atmospheres Containing Different Proportions of Carbon Dioxid, Others in Darkness*

| Organs of 28 Plants | Time of Year | Carbon Dioxid in Atmosphere | | | | Darkness |
|---------------------------------|------------------------------|-----------------------------|-------------------|-------------------|---------------------|-------------------|
| | | Approximately 1% | Normal Atmosphere | Very Small Amount | Approximately 0.02% | Normal Atmosphere |
| Leaves | Sept. 3-Oct. 5 Oct. 10-31 | N. in mg. 94 96 | N. in mg. 108 | N. in mg. 125 | N. in mg. 149 | N. in mg. 8 |
| Stems, hypocotyls, and petioles | Sept. 3-Oct. 5 Oct. 10-31 | 99 104 | 86 | 74 | 63 | 169 |
| Roots | Sept. 3-Oct. 5 Oct. 10-31 | 70 66 | 66 | 38 | 29 | 19 |
| Cotyledons, unused residue | Sept. 3-Oct. 5 Oct. 10-31 | 44 50 | 44 | 66 | 78 | 106 |

used in the calculations of the former experiments. It was found that 28 seeds contained approximately 328 mg. of nitrogen. An exact determination of the nitrogen content of the seeds was made after a preliminary analysis to find the number of seeds required to yield approximately 328 mg. of

nitrogen. The weights and nitrogen content of three lots of seeds of 28 seeds each were determined in the manner previously described (13). The following results were obtained:

| | I | II | III |
|--|------------------|------------------|------------------|
| Weight of seeds..... | 5.914 gm. | 5.838 gm. | 5.872 gm. |
| Percentage of nitrogen in duplicate samples of dry material..... | 1—5.57 2—5.59 | 1—5.62 2—5.55 | 1—5.63 2—5.53 |
| Total nitrogen in seeds..... | 330 mg. | 326 mg. | 328 mg. |

Difference between highest and lowest amounts found, 4 mg.

Greatest possible difference between different lots of seeds, 10 mg.

RESULTS

Experiment 1. September 13 to October 5, 1928

The seeds were placed in germinators September 8 and the seedlings were planted in sand September 13. Six cultures of eight plants each were grown in an atmosphere containing approximately 1 percent carbon dioxid, six similar cultures in an atmosphere supplied with 0.002 to 0.003 percent carbon dioxid, and six in darkness. Additional carbon dioxid was furnished to the high carbon dioxid cultures only during periods of sunshine, when light conditions were sufficiently favorable to permit the utilization of more carbon dioxid than is found in the normal atmosphere.

100 cc. of the nutrient solution were given to each of the cultures on the following days: September 17, 20, 24, 26, and October 1. Ammonia-free water was supplied at intervening times in amounts sufficient to maintain a layer of water about 1 cm. deep in the granite pans in which the porous pots rested.

The temperature of the greenhouse at night was kept at 21° C. During the daytime it sometimes went up to 27° C. Light and temperature conditions in the vicinity of Yonkers, N. Y., are usually favorable at this season of the year for this type of an experiment. The desired condition was brilliant illumination during the day without too much of a heating effect, a condition which would permit a rapid synthesis of carbohydrates. Unfortunately these conditions were not attained. The heavy walls of the bell jar apparatus and the film of moisture which frequently coated the inside of the jars reduced very appreciably the intensity of the light received by the seedlings. These two factors in addition to much cloudy weather combined to make light very definitely a limiting factor in carbohydrate synthesis. The long, slender stems of the seedlings were an evidence of etiolation effects. Notwithstanding the fact that conditions did not permit the maximum rate of carbohydrate synthesis there were definite differences in the growth and allocation of nitrogen in the different organs of the three groups of seedlings.

The results of this experiment will be mentioned briefly; those of experiment 2 will be described more fully. The most noticeable characteristics of the high carbon dioxid plants as compared with those grown in the lower concentration were larger root systems, longer and heavier stems, cotyledons larger and somewhat less green at an early stage, more rapid loss of their green color, and earlier yellowing of the leaves. The relative green and dry weights of the different organs of the three groups of plants may be observed in table 1 and the dimensions of the organs in table 2.

The first leaf of the high carbohydrate plants was larger than that of the low carbohydrate plants. Apparently the high concentration of atmospheric carbon dioxid furnished more favorable conditions for its growth. The second and third leaves of the low carbohydrate plants were larger, however, than those of the high carbohydrate plants. A larger number of the latter plants had the fourth leaf unfolded. Because of the unfavorable light conditions the amount of carbohydrates synthesized by the low carbon dioxid plants was somewhat inadequate for growth.

When the plants were harvested the leaves of the low carbohydrate seedlings were greener, appeared younger, and contained a larger amount of nitrogen than those of the high carbohydrate plants (table 1, column 9). Per square centimeter of surface, the leaf from the first node contained 0.092 mg. as compared to 0.060 in the corresponding leaf of the latter plants, and the leaf from the second node 0.115 mg. in contrast to 0.084 in that of the latter plants. The third leaves had areas more nearly alike but the total amount of nitrogen contained in them and the nitrogen per unit area of surface was greater in the leaves of the low carbohydrate plants. The latter plants grew more slowly and the fourth leaf was just unfolding at the time of harvest. The fourth leaf of the high carbohydrate plants was larger and contained more nitrogen. If the seedlings had grown for a longer time the fourth leaf of the low carbohydrate plants would undoubtedly have grown larger and would have withdrawn more nitrogen from the cotyledons.

The chloroplasts of the palisade tissue of the first or oldest leaf of the high carbohydrate plants had little green color left and contained little visible material other than the starch grains with which they were filled. Sections made of corresponding leaves of the low carbohydrate seedlings revealed on microscopic examination large, bright green, relatively dense chloroplasts, which contained no starch. Sections of the fixed and stained tissues showed that the chloroplasts contained more safranin-stainable material than those of the high carbohydrate leaves. Similar differences were found in the second leaves, although the degeneration of the chloroplasts of the second leaf of the high carbohydrate seedlings had not advanced so far as it had in the first leaf. The third leaves of the two sets of plants were not so different. The chloroplasts of the third leaf of the high carbohydrate seedlings were greener than those of the two older leaves of the same plants and the degenerative effects were not so noticeable.

The Nitrogen Balance

Slight losses in recovery of the nitrogen were found. The decreases ranged from 21 to 26 mg. from each of the three groups of seedlings. This amount is not large and possibly the balance is almost as close as one could expect with the methods employed. No nitrogen in gaseous form that could be absorbed by acid was given off by the seedlings grown in the light.

Experiment 2. October 10 to 31, 1928

Seedlings with radicles 1 to 2 cm. long were removed from the germinators where they had been placed October 6 and were planted in sand October 10. The nutrient solution was applied in the same manner as in the previous experiment. Four different lots of seedlings consisting of 6 cultures each and 6 plants² per culture were grown under the following environmental conditions: (a) in the light in an atmosphere containing a relatively high concentration of carbon dioxide (approximately 1 percent), (b) in the light in an atmosphere containing half the amount of carbon dioxide in the normal atmosphere (approximately 0.02 percent), (c) in the light in the normal atmosphere, (d) in darkness. Because of the low light intensity in experiment 1 the amount of carbon dioxide supplied to the plants grown in the lower concentration (0.002 to 0.003 percent) was inadequate to allow a sufficiently rapid or complete utilization of the reserve nitrogen. It was hoped that the concentration of carbon dioxide used in experiment 2 would allow the seedlings to manufacture enough carbohydrates to permit the utilization of the reserve nitrogen but without much excess of unused carbohydrate material.

The bell jar apparatus used for conditioning the atmosphere with respect to carbon dioxide was placed on the central bench in the greenhouse so as to obtain as much sunlight as possible. The arrangement of the cultures was such that the two sets of seedlings grown under the bell jars had approximately equal illumination. The night temperature of the greenhouse was the same and the day temperature approximately the same as in experiment 1. Although light conditions were somewhat better than they had been for the first experiment, light was still definitely a limiting factor in the synthesis of carbohydrates. The first additional carbon dioxide was given on October 14 and thereafter it was supplied during all periods of sunshine. In previous experiments there had been some indication that toxic effects might result from increasing the carbon dioxide concentration of the atmosphere during cloudy weather.

A and B. High and Low Carbon Dioxide Plants

The chief characteristic of the high carbon dioxide plants as compared to those of the low carbon dioxide plants were longer and heavier stems and hypocotyls, larger root systems, cotyledons less green in early stages of

² The calculations given in all of the tables are for 28 plants. The 8 additional plants in each group were used in the microchemical tests.

development, earlier yellowing of the cotyledons, smaller total leaf areas, slower rate of growth of all leaves except those at the first node, more rapid progress of degenerative changes in the leaves associated with a more rapid break-down of the plasma and chlorophyll content of the chloroplasts, with a resulting diminution in the nitrogen content of the leaves.

One week after the tops of the seedlings had emerged above the substrate, distinct differences in the color of the cotyledons of the two groups of plants grown in the culture chambers and receiving different amounts of carbon dioxide were observed; those receiving the lower concentration were a darker, bluer green. Similar, although much less noticeable, differences were observed in the leaves. The sizes of the two sets of cotyledons were the same.

Somewhat more definite differences in greenness of the cotyledons and leaves of the two groups of plants were observed on the two following days. The 28 low carbon dioxide plants had 52 leaves with blades open and exposed to the light, whereas the high carbon dioxide plants had only 40. Eleven days after the tops of the plants had grown above the level of the sand the plants grown in the lower concentration of carbon dioxide had 77 unfolded leaves and those in the higher concentration 62. The cotyledons of the former were still considerably darker green but at this time no differences in the color of the leaves were observable.

Two days later the low carbon dioxide plants had 95 unfolded leaves and the other plants had 80. At this time the cotyledons of almost all of the high carbon dioxide plants had a yellowish tinge in the green, a few had already lost most of their green color. The chlorophyll at first disappeared rather uniformly from all regions but in the later stages it tended to disappear somewhat more rapidly at the margins. The cotyledons of the low carbon dioxide plants did not exhibit a general yellowing previous to the disappearance of the chlorophyll. The loss of some of the chlorophyll resulted in a grey-green color. The complete disappearance of the chlorophyll occurred first at the lateral margins.

On the sixteenth day after the tops of the two groups of the plants had become exposed to the light the following observations were made of the color of the cotyledons:

High carbon dioxide plants:

- 11 plants had cotyledons almost entirely yellow, some beginning to wither.
- 10 plants had cotyledons partly yellowed.
- 5 plants had cotyledons yellow-green.
- 2 plants had cotyledons green.

Low carbon dioxide plants:

- 0 plants had cotyledons almost entirely yellow.
- 12 plants had cotyledons partly yellow.
- 4 plants had cotyledons greyish-green.
- 12 plants had cotyledons green.

At this time the high carbon dioxid plants had 126 open leaves and the low carbon dioxid plants 125 open leaves.

The appearance of the leaves of representative plants of the two groups of seedlings is shown in figures 2 and 3 in Plate XXXIV. The relative sizes of leaves from different nodes of the two types of plants are shown in table 5. The first leaf of the high carbon dioxid plants was larger than that of the low carbon dioxid plants. It appeared that after the development of the first leaf by the latter seedlings the photosynthetic area was adequate to provide sufficient carbohydrates for growth. The second, third, and fourth leaves became larger than those of the high carbon dioxid plants. At the end of the experimental period the leaves of the latter plants had about reached the limit of growth. The utilizable reserves were almost depleted from the cotyledons. If the low carbon dioxid plants had been allowed to grow until the reserves from the cotyledons had reached the same degree of depletion these plants might have had a larger number of leaves than the high carbon dioxid plants. The differences in total leaf areas of the two groups of plants would also undoubtedly have been greater.

During the intermediate phases of development the leaves of the high carbon dioxid plants usually appeared to be considerably greener in the morning than at the end of a sunshiny day. As the leaves became yellower this difference in chlorophyll at different times of day was not noticeable. The older leaves of the high carbon dioxid plants diminished in greenness markedly during the later stages of development. At the end of the experimental period the first, second, and third leaves of the high carbon dioxid plants were much less green and more yellow than those of the low carbon dioxid plants. There appeared to be a decrease in greenness from the youngest to the oldest leaves of the former plants. The first leaf of the low carbon dioxid plants was somewhat less green than the other leaves of the same plants, but it was noticeably greener than the first leaf of the high carbon dioxid plants. The other leaves of the former plants were all of about the same shade, a fresh green.

Sections of the first and third sets of leaves were examined microscopically. The cells of the third leaf of the high carbon dioxid plants were almost gorged with starch and contained large amounts of reducing substances. The first leaf also had a fairly high content of reducing substances but much less starch than the third leaf. At an earlier stage its starch content had been much higher. The chloroplasts of the palisade tissue of the first leaf showed a marked degeneration when the seedlings were harvested. The chlorophyll was nearly gone and the chloroplasts were fragmented. Even in the third leaf the amount of green substance in the chloroplasts seemed slight. In the leaves of the low carbon dioxid plants the chloroplasts were rounded, much larger, and bright green. The chloroplasts of the first or oldest leaf were slightly less green than those of the other leaves. There were very slight traces of starch and free-reducing substances in the leaves of these plants. A more exact description of the

cytological differences between the leaves of the two types of plants will be published later.

Leaves of the low carbon dioxide plants wilted very quickly when exposed to the air, whereas those of the high carbohydrate plants did not appear to do so. The veins of the latter plants were heavier and the petioles stouter. The yellowing cotyledons of the high carbohydrate plants retained their shape during the entire time that the chlorophyll was disappearing. Most of them appeared plump with no trace of withering when the visible cell contents were almost depleted except for the nucleus and some yellow globules, the chemical nature of which was unidentified. Although the cotyledons had previously contained large amounts of starch, only traces were present at this stage. Cotyledons of the low carbohydrate plants shriveled quickly in the yellowed places.

From the data given in table 5 and from the different appearance of the two types of seedlings as shown in figures 2 and 3, it may be noted that the high carbohydrate plants had longer stems and hypocotyls than the low carbohydrate plants. The results show that synthesis of carbohydrates favors the growth of stems and hypocotyls.

Strengthening tissues of the stems and hypocotyls, but particularly of the stems, were much more developed in the plants having an abundance of carbohydrates. The stems of the low carbon dioxide plants were greener and the surface was smoother because the bundles were not sufficiently developed to cause the conspicuous ridges noted in those of the high carbon dioxide plants. The stems and hypocotyls of the latter plants contained exceedingly large amounts of starch and free-reducing substances. Similar tissues of the low carbon dioxide plants contained only traces of reducing substances and starch. Figures 5 and 6 of Plate XXXV are photomicrographs of cross sections of the hypocotyls of seedlings grown in high and low concentrations of carbon dioxide, respectively. The iodine test was used to show the difference in starch content. Figures 7 and 8 of Plate XXXV show the differences in content of reducing substances (Flückiger reaction) in the hypocotyls of seedlings grown in the high and low concentrations of carbon dioxide, respectively.

The roots of the high carbon dioxide seedlings were long, very numerous, and much branched. The plants had adventitious roots growing from the hypocotyls, a development probably favored by the moist atmosphere of the culture chambers. The plants grown in the lower concentration of carbon dioxide had fewer roots, they were less branched, and somewhat shorter than those of the high carbohydrate plants. None of these plants developed adventitious roots.

Allocation of Nitrogen in Different Organs of the Seedlings

In column 7 of table 3 it may be observed that the cotyledons of the low carbohydrate plants contained more of a residue of nitrogen than those of the high carbon dioxide plants.

The process of carbohydrate synthesis has marked effects upon the amounts of nitrogen found in the leaves. The leaves of the seedlings which were permitted to synthesize only a small amount of carbohydrates contained 149 mg. of nitrogen; those of the seedlings which synthesized relatively large amounts of carbohydrates contained only 96 mg. of nitrogen. The data of column 9 of table 3 show the amount of nitrogen per unit area of leaf surface of the leaves from different nodes of plants grown under conditions of high and low carbohydrate synthesis. The amount of nitrogen per unit area of leaf surface of the seedlings which synthesized an abundance of carbohydrates was highest in the youngest leaf and lowest in the oldest leaf, the leaves at intervening nodes having intermediate amounts according to their age and position on the stem.

It is probable on the basis of evidence obtained in previous experiments (14) that the nitrogen content of the first, second, and third leaves of the high carbon dioxid seedlings was relatively high during the early and intermediate stages of their development, but diminished during the later stages. The supposed loss of nitrogen during the later phases of development of the leaf is correlated with the previously described diminution of chlorophyll and plasma content of the chloroplasts.

The leaves of the seedlings grown in the lower concentration of carbon dioxid, most of which appeared young and a fresh green at the time of harvesting and whose plastids were much larger, well rounded, and had a denser plasma content, contained much more nitrogen per unit area of surface than leaves of the same age of the high carbon dioxid seedlings. There appeared to be some loss of nitrogen and chlorophyll from the first leaf possibly resulting from too great a limitation of carbohydrates.

The plants which synthesized an abundance of carbohydrates contained more nitrogen in both stems and hypocotyls than those of the low carbohydrate seedlings. The synthesis of carbohydrates apparently affects the amount of nitrogen found in the stem structures. The higher content of nitrogen in the stems of seedlings synthesizing an abundance of carbohydrates may result chiefly from nitrogen translocated from the leaves.

The roots exhibited remarkable variations in the amount of nitrogen present under conditions of high and low carbohydrate synthesis. The roots of the seedlings grown in the higher concentration of carbon dioxid contained 66 mg. of nitrogen; those of the seedlings grown in the low concentration of carbon dioxid contained only 29 mg. One of the most interesting features to be observed in connection with the nitrogen content of the roots is that the percentage of nitrogen in their green weight was approximately the same under the two conditions for growth (table 3).

The Nitrogen Balance

An almost complete recovery of the nitrogen was obtained from the two groups of seedlings grown in high and low concentrations of carbon dioxid.

No nitrogen in gaseous form that could be absorbed by acid was given off by the seedlings of either group.

C. Seedlings Grown in the Open, in the Greenhouse, and in the Normal Atmosphere

The cotyledons, leaves, and roots of these plants had many of the same general characteristics as those of the seedlings grown in the bell jar chambers in the high concentration of carbon dioxid. The stems, however, were much shorter, undoubtedly because of the higher light intensity. One of these seedlings is shown in figure 4. The first leaf was larger than the first leaf of either of the two groups of seedlings grown in the bell jar chambers. Its growth was apparently favored by the higher light intensity. The average size of the remaining leaves was much less than that of seedlings grown in the low concentration of carbon dioxid. After the first leaf of these plants had attained full size there was an interval of considerable length during which there was a very slow rate of initiation and growth of younger leaves. After the first leaf began to show evidences of decline the rate of growth of the younger leaves was notably accelerated. There was some evidence from earlier experiments (14) that leaves of seedlings grown without an external supply of nitrogen and under conditions which permitted a fairly rapid synthesis of carbohydrates do not gain appreciably in nitrogen content after reaching maximum size. During the period of very slow growth of younger leaves the nitrogen released from the cotyledons and available for leaf growth was probably used by the first or oldest leaf for purposes of maintenance.

The relative sizes of the organs of the different groups of seedlings of this experiment are shown in table 5.

The leaves of the plants grown in the normal atmosphere were exposed to light of higher intensity and to a less humid atmosphere than those of the seedlings grown in the culture chambers. The leaves were thicker and had a more compact structure. These leaves consequently had a higher content of nitrogen per unit area of surface than those of the seedlings grown in the bell jar chambers. The first or oldest leaves were the only ones which showed definite evidences of yellowing. There may have been some loss of green color from the second leaves but it was relatively slight. The nitrogen content of the first leaves undoubtedly diminished during the later stages of development, the same as those of the seedlings similarly grown in a previous experiment (14). The amount of nitrogen found in all of the leaves of the 28 seedlings was 108 mg. The leaves similar to those of the high carbon dioxid seedlings did not wilt quickly when detached from the plants. The high content of starch and free-reducing substances and the loss of chlorophyll and plasma from the chloroplasts of the older leaves were similar to conditions in the leaves of the high carbon dioxid seedlings, although the degenerative processes were somewhat more advanced in the

leaves of the latter plants. It is supposed that the higher intensity of the light received by the plants grown in the open compensated for the lower concentration of carbon dioxid with the result that the plants grown in the open in the normal atmosphere synthesized approximately as much carbohydrate material.

The stems and hypocotyls of the seedlings grown in the normal atmosphere were shorter than those of the seedlings grown in the culture chambers. They contained large amounts of starch and free-reducing substances. More nitrogen was found in the stem structures than in those of the low carbon dioxid plants but less than that found in similar tissues of the high carbon dioxid plants. This may be because of the fact that degenerative changes in the leaves of the former plants had proceeded farther.

The roots of these seedlings appeared much like those of the seedlings grown in the high concentration of carbon dioxid (figs. 3 and 4), although both the green and dry weights of the former were slightly higher. The amount of nitrogen found in the roots of the two groups of seedlings was the same.

There was a slight loss (24 mg.) in the total amount of nitrogen recovered from these seedlings.

D. Seedlings Grown in Darkness

The cotyledons of these seedlings were much smaller and the green and dry weights were less than those of any of the three groups of seedlings grown in the light. A slight trace of reducing substance, no starch, but considerable fat was found in them at the end of the experimental period.

Only part of the seedlings developed a leaf at the first node and these remained small and whitish. No starch was found in the leaves. The guard cells contained plump greenish plastids.

These plants had exceedingly long hypocotyls, but the stems were very short, probably because so much of the food reserves had been used in the growth of the hypocotyls. These plants had the smallest root systems of the four groups of plants grown in this experiment. The roots were shorter, fewer, and less branched than those of the low carbohydrate plants grown in the light. Figure 1 represents one of these seedlings.

Only a few milligrams of nitrogen were found in the leaves of these seedlings. The hypocotyls contained about 300 percent more nitrogen than those of the high carbon dioxid seedlings and about 500 percent more than those of the low carbon dioxid seedlings. The roots of these seedlings contained only 17 mg. of nitrogen. The roots were somewhat flaccid when the plants were harvested and there may have been some loss of nitrogen from them. There was a discrepancy in the nitrogen balance of the plants of 45 mg.

DISCUSSION

Since the squash seed has a high nitrogen content, the seedlings require the addition of considerable extra carbon in order to allow a relatively complete utilization of the reserve nitrogen. The availability of only small amounts of carbon dioxide limits the growth of stems and roots especially. These results are in general agreement with those obtained in previous investigations (11). A number of additional observations have also been made. Comparisons of the effect of increasing the carbon dioxide content of the atmosphere above that of the normal atmosphere cannot be made from the results here reported since light conditions were somewhat different for the seedlings grown under these two sets of conditions.

There is some evidence that the amount of nitrogen found in the stem structures of seedlings grown in the light may be related to the carbohydrate content of the tissues and to the physiological condition of the leaves. If the leaves show degenerative effects accompanied by loss of nitrogen, an accumulation of nitrogen in the stems may occur. The movement of nitrogen into the stems from leaves yellowing as a result of a shortage of nitrogen and an accumulation of carbohydrates is in line with the increase of nitrogen in stems following autumnal yellowing of the leaves as reported by Combes (5) and other investigators.

The differences in total leaf area between the low and high carbohydrate seedlings may be a consequence of various influences: (a) The utilization of less nitrogen by the roots during the early phases of growth may have left more available for the growth of leaves of the low than in the case of the high carbohydrate seedlings; (b) Some of the nitrogen translocated from the cotyledons and available to the leaves was used by the fully grown leaves for replacement of the nitrogen compounds broken down during periods of active photosynthesis. The production of a very small total leaf area by seedlings grown in the normal atmosphere during June, 1927 (13) could thereby be explained. This hypothesis would also help to account for another peculiarity in the growth of high and low carbohydrate seedlings, *i.e.*, the more rapid development of leaves by the low carbohydrate seedlings during the early and intermediate phases of growth. The leaves of these seedlings required none or at least only a little nitrogen for reconstructive purposes, all or almost all that was available for leaves could be used for the initiation, growth, and maturing of new leaves. In the later phases of development of the high carbohydrate plants the older leaves instead of drawing on the reserve supply of nitrogen were releasing nitrogen in consequence of the rapid progress of degenerative processes. Conditions may, therefore, have then been more favorable than during the previous period for the growth of the younger leaves; (c) The nitrogen compounds broken down in the foliaceous tissues in association with carbohydrate synthesis were in part or wholly unsuitable for further use by leaves. Spoehr's suggestion that chlorophyll is not successively decomposed and re-formed is in line with this conception.

The difference in intensity of greenness during the early phases of growth of cotyledons of seedlings grown in high and low concentrations of carbon dioxide, those of the latter plants being greener; the differences in the character of the green color of the two sets of cotyledons during the intermediate phases of growth, those of the high carbohydrate plants tending to become yellow-green and those of the low carbohydrate plants a grey-green, indicate differences in the nitrogen metabolism under the two sets of conditions.

From these results as well as from others previously obtained it appears that an abundant synthesis of carbohydrates under conditions of limited nitrogen supply results in a final decrease in the size of the chloroplasts of the palisade tissue, the relatively early loss of some of the chlorophyll, and also some of the plasma contained in the chloroplasts. The changes proceed somewhat slowly at first. During days of intense photosynthesis there may be a loss of chlorophyll but a renewal apparently occurs at night or by early the following morning. Because of the small number of plants that could be grown it was not possible to make quantitative determinations of the pigments and protein content but it is hoped that investigations of this kind may be made in later experiments. The daily changes in color of leaves appear to be similar to those reported by Henrici (7) with respect to the chlorophyll content of grasses.

Microchemical analyses indicated that the period of noticeable breakdown of the plasma and chlorophyll of the chloroplasts is associated with a decrease in starch and an accumulation of reducing substances. Smirnow's results (17) showed that during the periods in which the reducing substances were present in largest amounts per unit area of leaf surface the protein content and starch tended to be lowest, and during the periods in which the reducing substances were present in smallest amounts the protein and starch content tended to be the highest. Since there is considerable evidence that the plasma of the chloroplasts consists largely of protein or a protein-complex the relations observed in the case of squash seedlings as herein described may be comparable to those in Smirnow's experiments.

The differences in greenness of cotyledons and leaves of the high and low carbon dioxide squash seedlings during the early phases of growth may indicate that the availability of a relatively large amount of the active forms of carbohydrates in proportion to the available nitrogen produces conditions unfavorable for the synthesis of nitrogenous substances contained in the chloroplasts. Somewhat later, the decrease in the chlorophyll and plasma content of the chloroplasts following periods of active photosynthesis indicates a failure in the maintenance of these chloroplastid substances. Whether the process of photosynthesis has a directly destructive effect on the chloroplasts as postulated by Sachs (16) or whether all of the degenerative changes are indirectly produced by transformations occurring in connection with an accumulation of reducing sugars or some other active type of compound cannot be stated from our present knowledge of the problem.

SUMMARY

A. Effect of high (1 percent) as compared to low (0.02 percent) concentrations of carbon dioxid:

1. On growth.

Increased green and dry weights of organs; longer, heavier stems; much larger root systems; smaller total leaf areas; blades of all leaves except those at first node smaller in size; slower rate of production of leaves until toward the end of the growing period; greater development of the strengthening tissues of the stems and leaves.

2. On carbohydrate content of tissues.

Larger amounts of starch and reducing substances in all tissues; large amounts of starch and reducing substances in leaves of the high carbon dioxid plants when degenerative changes in the chloroplasts were first observed. Later in the more advanced stages of decomposition, the starch decreased rapidly.

3. On chlorophyll content.

Cotyledons less green in early stages of development. Similar, although less pronounced, differences observed in the first two sets of leaves.

Earlier break-down of chlorophyll in the cotyledons resulting in a yellow-green and finally a yellow color. Cotyledons of the low carbon dioxid plants became grey-green and eventually yellow.

Decrease in the chlorophyll content of the leaves became apparent relatively early, the oldest leaves showing the effects first; those of seedlings grown in the low concentration of carbon dioxid showed no such changes except to a slight extent in the case of the first leaf.

4. On plasma content of chloroplasts.

More rapid break-down of plasma in chloroplasts of cotyledons than in those of the low carbon dioxid plants.

Associated with changes in color of the leaves as they became older there was a decrease in the plasma content of the chloroplasts. Such changes were not observed in the leaves of the low carbon dioxid plants, except to a slight extent in the leaf at the first node.

5. On allocation of nitrogen.

More of the total nitrogen was found in roots and stems of the seedlings grown in the higher concentration of carbon dioxid.

Less of the total nitrogen was found in the leaves than in those of seedlings grown in the lower concentration of carbon dioxid.

The leaves from the first, second, and third nodes had much smaller content of nitrogen per unit area of leaf surface than corresponding leaves from the low carbon dioxide plants. If these results are interpreted in consideration of findings in previous experiments, it would appear that the lower content of nitrogen of the older leaves of the high carbon dioxide plants had resulted as a consequence of the degenerative processes such as decrease in chlorophyll and plasma content of the plastids.

B. Effects of light other than in carbohydrate synthesis upon growth and nitrogen metabolism of the seedlings.

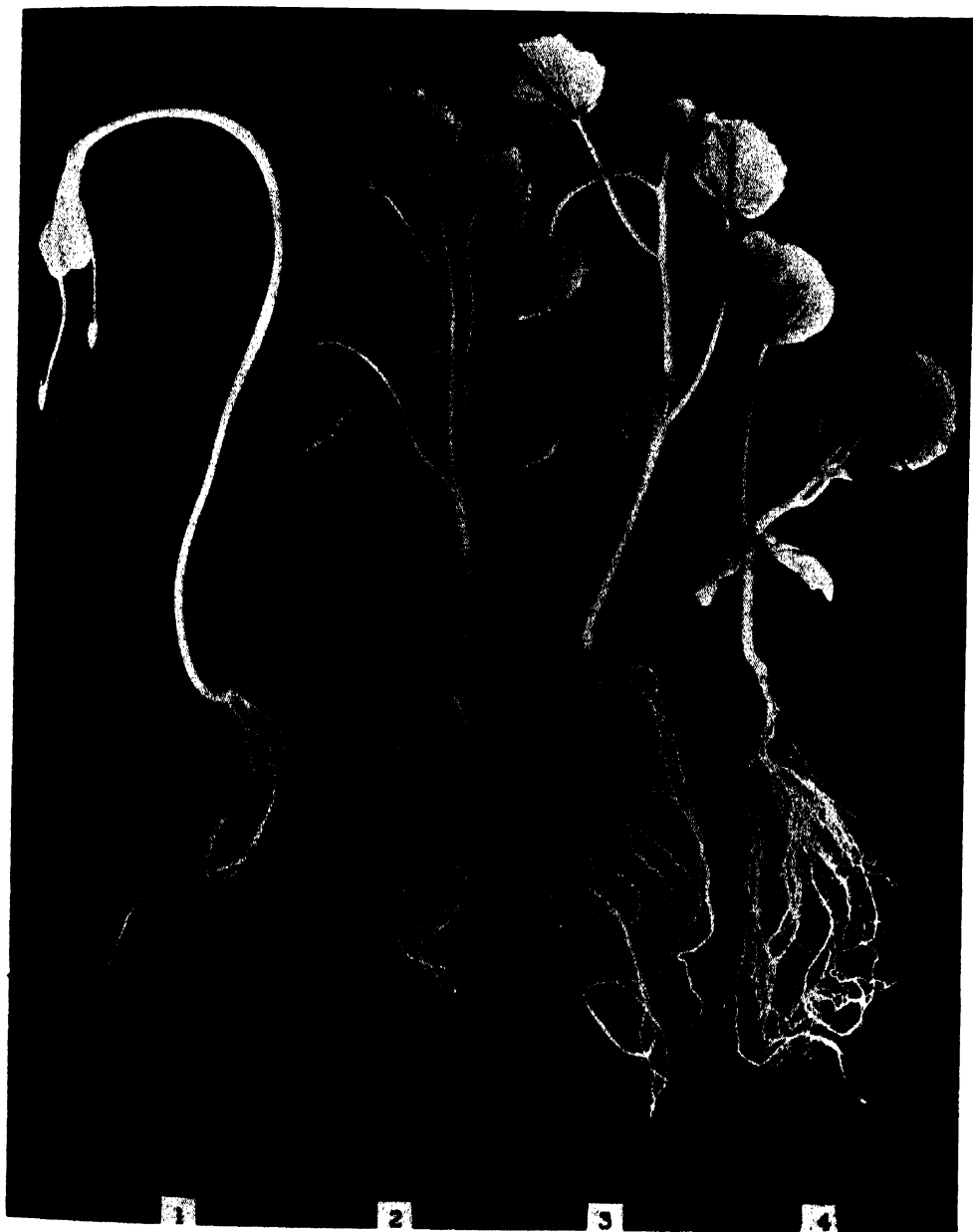
1. Seedlings grown in light have shorter hypocotyls which contain less nitrogen than those of seedlings grown in darkness.
2. Light favors the growth of leaves. It produces great differences in number, size, and color of the leaves, and also in the amount of nitrogen found in them.

C. Seasonal differences in growth and utilization of the reserve nitrogen may be accounted for in many respects by differences in the amounts of total carbohydrates, or by differences in the amounts of certain types of carbohydrates synthesized at opposite seasons of the year (June and December). The longer stems of December plants, however, are a result of other influences of light.

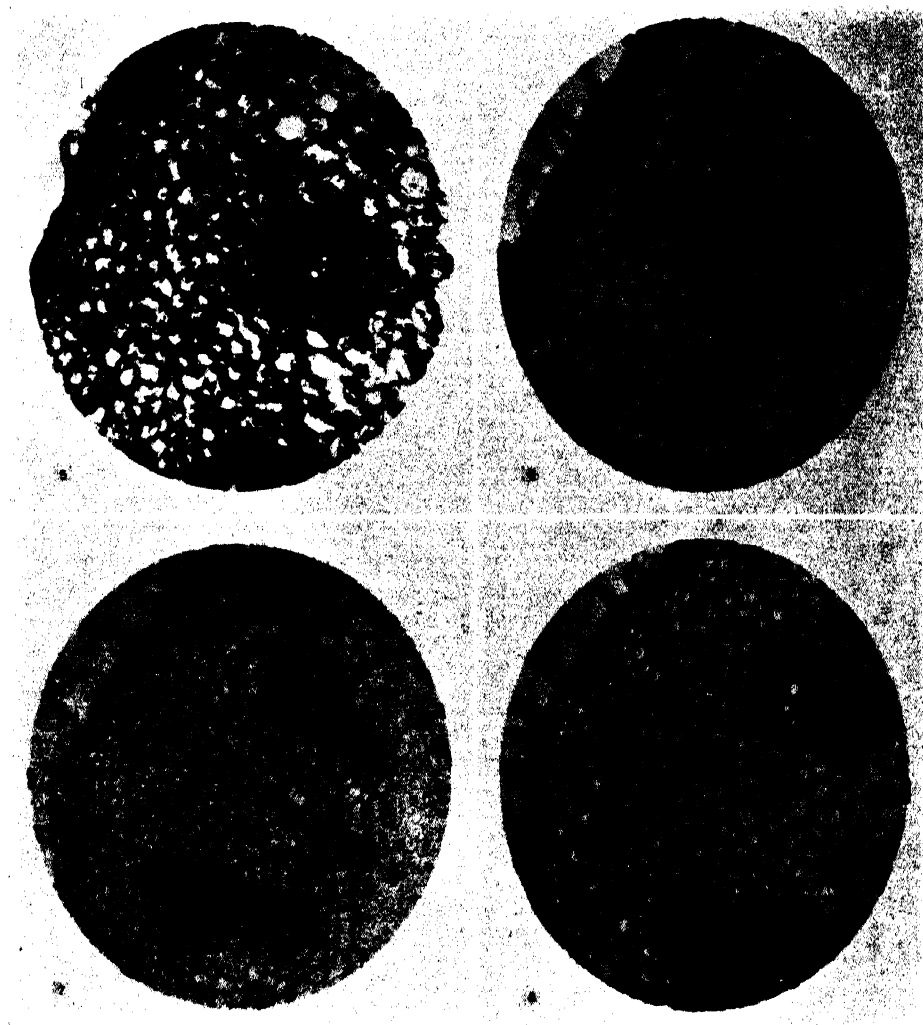
I wish to express appreciation of the encouragement and helpful criticism of Professor Lafayette B. Mendel during the progress of the investigations and of many courtesies extended by Professor Arthur H. Smith and by members of the Botanical Department of Yale University. I also desire to make grateful acknowledgment to the Connecticut Agricultural Experiment Station for facilities employed in connection with the culture of the plants and to the Boyce Thompson Institute for Plant Research for the photographs used in illustrating these papers and for the use of special apparatus. Thanks are also due to Mr. John M. Newell for making the determinations of the carbon dioxide content of the atmospheres in which some of the seedlings were grown.

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REID: NITROGEN METABOLISM



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ALGAE COLLECTED BY THE HASSLER, ALBATROSS, AND SCHMITT EXPEDITIONS

I. MARINE ALGAE FROM BRAZIL

WM. RANDOLPH TAYLOR

(Received for publication February 22, 1930)

For some years the writer has had in hand material from South America collected by various expeditions, identification of which has been delayed for various reasons. Some progress having been made in the study of it, the writer now proposes to report upon a first portion, dealing with the specimens from three sources together as they derived from the several areas in which collections were made. It happens that the voyages of the "Hassler" and the "Albatross" and the two collecting trips of Dr. Schmitt brought material from such similar districts that a combined presentation is possible. A history of these collections from a phycological standpoint is best left to a concluding paper, since portions only have been studied, but their bulk and the territory involved can be briefly designated as follows:

Hassler Expedition. This expedition in the Coast Survey steamer "Hassler" was under the scientific direction of Louis Agassiz. It left Boston, Massachusetts, on December 4th, 1871, and spent most of March 1872 in the Straits of Magellan, reaching San Francisco, California, near the end of August 1872. Considerable collections of biological material were made at a number of places, but the algae appear to have been a quite minor incident of the trip. According to Agassiz they were collected by Thomas Hill, a physicist and former president of Harvard (1). They were usually rather roughly prepared, different kinds being mounted indiscriminately together on coarse paper, without very complete data, but many samples were in fair quantity. A general description of the trip will be found in Agassiz's biography (2).

Albatross Expedition. This expedition in the Fisheries' Commission steamer "Albatross" was under the scientific direction of L. H. Lee, assisted by C. H. Townsend, Thomas Lee, and D. M. Cole. No large assortment of marine algae was collected, but about 235 specimens reached the writer's

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hands, from 14 stations, some species with several duplicates. Collections were made off Pernambuco in December 1887, in the Straits of Magellan during January 1888, and in the Lower California area in April-May 1888. An account of the expedition will be found in the Reports of the Commission (4).

Schmitt Expeditions. The algae listed for convenience under this title were collected on two trips taken by Dr. Waldo L. Schmitt on the Walter Rathbone Bacon Scholarship during 1925-27. This material was collected in bulk and shipped in formalin either in bottles, jars, or 5-gallon tins. The quantity was great, and the sorting and mounting consumed much time, but the result was a very large amount of useful material, fortified by the reserve specimens kept in fluid. Since the formalin was in many cases not exposed to the light even delicate Ceramiaceae survived remarkably well both in texture and coloring, and the collection is expected to prove of exceptional value, even though the number of stations represented is small.

For the opportunity to study the material from the "Hassler" and the "Albatross" the writer is indebted to the Farlow Herbarium of Cryptogamic Botany (Harvard University) and its curator, Dr. Carroll W. Dodge. For the other collection grateful acknowledgment is made of the kindness of the collector, Dr. Waldo L. Schmitt, and also of Mr. W. R. Maxon of the National Herbarium. Dr. Marshall A. Howe, of the New York Botanical Garden, kindly consented to determine several difficult specimens, and to describe certain new ones (3).

ALGAE OF THE GULF STREAM

Lacking a more appropriate place in this series of reports, mention may be made here of some collections of *Sargassum* by the Hassler Expedition before reaching the coast of Brazil. It appears that these came from west of the Bermudas, in the Gulf Stream, during the observations described on pages 698-703 of the Biography (2).

SARGASSUM FLUITANS Børg.—N. Lat. $32^{\circ} 30'$, W. Long. 69° ; N. Lat. 30° , W. Long. 69° . Both the usual wide-leaved type, and a more unusual narrow one, were secured.

SARGASSUM NATANS (L.) Meyen.—N. Lat. $32^{\circ} 30'$, W. Long. 69° ; N. Lat. 30° , W. Long. 69° . Splendid specimens, perfectly typical.

SARGASSUM VULGARE C. Agardh.—N. Lat. 30° , W. Long. 69° . Large, sterile plants.

LIST OF BRAZILIAN ALGAE

Myxophyceae

TRICHODESMIUM THIEBAUTHII Gomont.—*Hassler*: floating off Bahia.

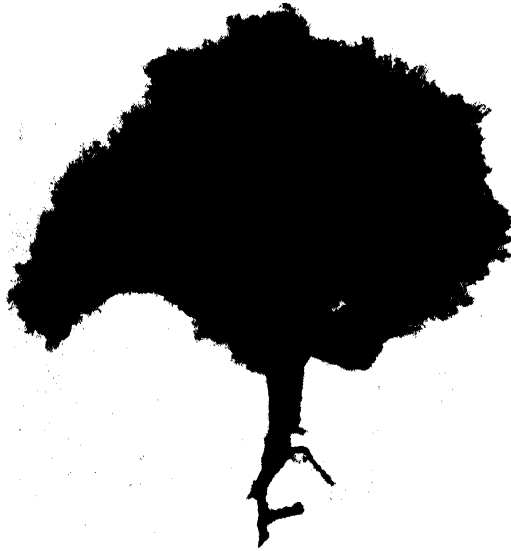
Chlorophyceae

ENTEROMORPHA FLEXUOSA (Wulf.) J. Ag.—*Hassler*: Harbor of Rio Janeiro.
ULVA FASCIATA Delile.—*Schmitt*: Conti de Rio, Nictheroy, near Rio Janeiro.

- ULVA LACTUCA L., var. LATISSIMA (L.) DC.—*Hassler*: Harbor of Rio Janeiro.
- ULVA LACTUCA L., var. RIGIDA (C. Ag.) LeJolis.—*Schmitt*: Pedra de Itapuca, Nictheroy; Praia Ensenada, São Francisco do Sul, Santa Catherina; Villa Bella, Ilha São Sebastião, São Paulo.
- CLADOPHORA FASCICULARIS (Mont.) Kg.—*Schmitt*: Villa Bella, Ilha São Sebastião.
- DASYCLADUS VERMICULARIS (Scop.) Krasser.—*Hassler*: Cabo Frio, dredged.
- ANADYOMENE STELLATA (Wulf.) Ag.—*Hassler*: Near the Abrolhos Ids, dredged; *Albatross*: Off Pernambuco, dredged in 20 fathoms.
- CHAMAEDORIS PENICULUM (E. & S.) Ktze.—*Hassler*: Near the Abrolhos Ids, dredged; near Cabo Frio, dredged. *Albatross*: Off Pernambuco, dredged in 20 fathoms.
- DICTYOSPHAERIA FAVUOSA (C. Ag.) Dcne.—*Hassler*: Off Cabo Frio, dredged.
- VALONIA VENTRICOSA J. Ag.—*Hassler*: Near the Abrolhos Ids, one plant dredged.
- CAULERPA ASHMEADII Harv.—*Albatross*: Dredged off Pernambuco in 20 fathoms. Large pieces, in perfect condition.
- CAULERPA CRASSIFOLIA (C. Ag.) J. Ag., var. MEXICANA (Sond.) J. Ag.—*Hassler*: Near the Abrolhos Ids., dredged.
- CAULERPA LANUGINOSA J. Ag.—*Albatross*: Dredged off Pernambuco in 20 fathoms. A large and sturdy form of the species.
- CAULERPA RACEMOSA (Forsk.) J. Ag., ?var. CLAVIFERA (Turn.) Web.—*Schmitt*: Pedra de Itapuca, Nictheroy. A very small form.
- CAULERPA RACEMOSA (Forsk.) J. Ag., ?var. LAETEVIRENS (Mont.) Weber.—*Schmitt*: Villa Bella, Ilha São Sebastião. Ramuli usually expanded at the summit and so tending towards var. *occidentalis*, but too crowded for that variety, and wrongly shaped for var. *uvifera*. Leafy branches to 13 cm. tall.
- CAULERPA RACEMOSA (Forsk.) J. Ag., var. OCCIDENTALIS (J. Ag.) Børg.—*Hassler*: Scarahy, Rio Janeiro.
- CAULERPA RACEMOSA (Forsk.) J. Ag., var. UVIFERA (Turn.) Weber.—*Schmitt*: Villa Bella, Ilha São Sebastião.
- CAULERPA SERTULARIOIDES (Gmel.) Howe.—*Schmitt*: Villa Bella, Ilha São Sebastião.
- CODIUM sp.—*Hassler*: Dredged off Cabo Frio. Small, encrusting. Perhaps only a juvenile disk of *C. decorticutum*; utricles to $425\ \mu$ diameter.
- Species of the genus *Codium* were not as clear-cut in the Brazil material as had been expected. Difficulty was experienced in assigning some small specimens to *C. decorticutum* or *C. isthmocladum*, since the flattening was slight and they became very thin and nitent when dry, although generally dark in color.
- CODIUM DECORTICATUM (Woodw.) Howe.—*Hassler*: Cabo Frio, dredged; Scarahy, quite typically flattened in part, the rest subcylindrical, large, dark green, thin and nitent when dry. *Schmitt*: Ilha Paqueta near Rio Janeiro, Pedra de Itapuca, Nictheroy; Villa Bella, Ilha São Sebastião, Ilha São Francisco.
- ?CODIUM PILGER I O. C. Schmidt. *Schmitt*: Villa Bella, Ilha São Sebastião. Utricles to $400\ \mu$ in diameter, end wall rounded, thick, reaching $38\ \mu$ but generally $10-15\ \mu$. Plants thick and stiff when dry, not adhering to paper, branching fairly appropriate to this species.
- HALIMEDA TUNA (E. & S.) Lamx.—*Hassler*: Dredged near the Abrolhos Ids.; dredged off Cabo Frio.

RHIPHILIA TOMENTOSA Kg.—Text figure 1.—*Hassler*: Dredged off Cabo Frio. Plants to 10 cm. broad and 7 cm. tall, the base of the stipe being broken away; the spine-tipped branches of the flabellar filaments were quite distinct.

UDOTEA CYATHIFORMIS Decne.—*Hassler*: Dredged near the Abrolhos Ids. Plants of fair size, papery-thin and funnel-shaped.



TEXT FIG. 1. *Rhiphilia tomentosa*, smaller specimen. The measurements were taken from larger material, but this individual showed greater symmetry and more complete stalk and holdfast portions. The flabellar tuft was loosely spongy above to such an extent that the white mounting paper shows through between the filament. Natural size.

Phaeophyceae

ECTOCARPUS sp.—*Hassler*: Dredged near the Abrolhos Ids. *Schmitt*: Villa Bella, Ilha São Sebastião. Not in condition for specific determination.

COLPOMENIA SINUOSA (Roth) D. & S.—*Schmitt*: Pedra de Itapuca, Nictheroy; Villa Bella, Ilha São Sebastião.

HYDROCLATHRUS CLATHRATUS (Bory) Howe.—*Hassler*: Scarahy. *Schmitt*: Villa Bella, Ilha São Sebastião.

SPOROCHNUS BOLLEANUS Mont.—*Hassler*: Dredged near the Abrolhos Ids; dredged off Cabo Frio, juvenile, the apical tuft of filaments large.

SPOROCHNUS PEDUNCULATUS (Huds.) C. Ag.—Dredged off Cabo Frio; with rather tiny ramuli, but this is often the case of ramuli from the basal parts.

?*DICTYOTA BARTAYRESII* Lamx.—*Hassler*: Dredged off Cabo Frio.

DICTYOTA CERVICORNIS Kg.—*Hassler*: Dredged off Cabo Frio. *Schmitt*: Villa Bella, Ilha São Sebastião.

DICTYOTA DENTATA Lamx.—*Hassler*: Dredged off Cabo Frio. *Albatross*: dredged off Pernambuco in 20 fathoms.

DICTYOTA DIVARICATA Lamx.—*Hassler*: Dredged off Cabo Frio.

DILOPHUS ALTERNANS J. Ag.—*Schmitt*: Ilha Govenador, near Rio Janeiro; main branches to 3 mm. wide just above the forkings, so the determination fairly clear.

NEUROCARPUS DELICATULUS (Lamx.) Ktze.—*Schmitt*: Pedra de Itapuca, Nictheroy; Villa Bella, Ilha São Sebastião.

Material from 2 stations fitted perfectly the descriptions of *N. Hauckianus* (Möb.) Ktze., but that species name must disappear into the synonymy of *N. delicatulus*. Through the kindness of M. R. Meslin of Caen and of M. E. Chemin of Paris the writer has been enabled to examine the type of *N. delicatulus* and other material which had been examined by E. Bornet, and agrees with the latter that the marginal veins which are cited as the most important distinguishing feature of *N. Hauckianus* are present in the type material of the earlier described species.

NEUROCARPUS JUSTII Lamx.—*Hassler*: Dredged off the Abrolhos Ids; dredged off Cabo Frio.

PADINA SANCTAE-CRUCIS Børg.—*Hassler*: Dredged near the Abrolhos Ids.

PADINA VICKERSIAE Hoyt.—*Hassler*: Harbor of Rio Janeiro. *Schmitt*: Harbor and Ilha Govenador at Rio Janeiro; Villa Bella, Ilha São Sebastião.

ZONARIA VARIEGATA (Lamx.) C. Ag.—*Hassler*: Dredged off the Abrolhos Ids.; dredged off Cabo Frio; harbor of Rio Janeiro. *Albatross*: Dredged off Pernambuco in 20 fathoms.

ZONARIA ZONALIS (Lamx.) Howe.—*Hassler*: Dredged off Cabo Frio; harbor of Rio Janeiro. *Albatross*: Dredged off Pernambuco in 20 fathoms.

SARGASSUM CYMOSUM C. Ag.—*Hassler*: Scarahy. *Schmitt*: Pedra de Itapuca, Nictheroy; Paqueta and bay, Rio Janeiro; Villa Bella, Ilha São Sebastião.

SARGASSUM FILIPENDULA C. Ag.—*Hassler*: Harbor of Rio Janeiro. Large leaved type, the leaves to 5 cm. long, 13 mm. wide, with spines projecting 1.5 mm. further on each side.

Rhodophyceae

GALAXAURA CYLINDRICA (E. & S.) Lamx.—*Hassler*: Dredged off Cabo Frio.

GALAXAURA MARGINATA (E. & S.) Lamx.—*Schmitt*: Ilha Paqueta near Rio Janeiro; Villa Bella, Ilha São Sebastião.

GALAXAURA OBLONGATA (E. & S.) Lamx.—*Schmitt*: Villa Bella, Ilha São Sebastião.

GELIDIUM CORNEUM (Huds.) Lamx.—*Schmitt*: Pedra de Itapuca, Nictheroy.

GELIDIUM RIGIDUM (Vahl.) Grev.—*Hassler*: Dredged off Cabo Frio.

CALLOPHYLLIS MICRODONTA (Grev.) Falk.—*Hassler*: Dredged off Cabo Frio. Det. M. A. Howe.

GYMNOGONGRUS GRIFFITHISIAE (Turn.) Mart.—a variety?—*Schmitt*: Ilha Paqueta. With *Actinococcus* sp? Det. M. A. Howe.

?EUCHEUMA GELIDIUM J. Ag.—*Schmitt*: Ilha Paqueta. A small piece, the microscopic structure suggesting this genus.

RHODOPHYLLIS GRACILARIOIDES Howe & Taylor.—*Hassler*: Dredged off Cabo Frio.

WURDEMANNIA SETACEA Harv.—*Hassler*: Dredged off Cabo Frio. *Schmitt*: Pedra de Itapuca, Nictheroy and Ilha Govenador.

GRACILARIA CONFERVOIDES (L.) Grev.—*Hassler*: Dredged off the Abrolhos Ids.

- GRACILARIA FEROX J. Ag.—*Hassler*: Scarahy and Harbor, Rio Janeiro. *Schmitt*: Villa Bella, Ilha São Sebastião. Fine, large plants; rather variable.
- GRACILARIA MAMILLARIS (Mont.) Howe.—*Hassler*: Dredged near the Abrolhos Ids; dredged off Cabo Frio.
- HYPNEA CERVICORNIS J. Ag.—*Hassler*: Harbor of Rio Janeiro.
- HYPNEA MUSCIFORMIS (Wulf.) Lamx.—*Hassler*: Harbor of Rio Janeiro. *Schmitt*: Villa Bella, Ilha São Sebastião.
- HYPNEA SPINELLA (C. Ag.) Kg.—*Hassler*: Scarahy. *Schmitt*: Villa Bella, Ilha São Sebastião.
- CHRYSYMENIA ENTEROMORPHA Harv.—*Hassler*: Dredged off Cabo Frio.
- CHRYSYMENIA PLANIFRONS (Melv.) J. Ag.—*Hassler*: Dredged off Cabo Frio. Det. M. A. Howe.
- CHRYSYMENIA PYRIFORMIS Børg.—*Hassler*: Dredged off Cabo Frio. The vesicles reached a length of 2.7 cm., the inner surface showing the typical clustered glands; part of the material was cystocarpic.
- FAUCHEA HASSLERI Howe & Taylor.—*Hassler*: Dredged off Cabo Frio.
- PLOCAMIAM BRASILIENSE (Grev.) Howe & Taylor.—*Hassler*: Dredged off Cabo Frio.
- PLOCAMIAM COCCINEUM Grev.—*Schmitt*: Pedra de Itapuca, Nictheroy.
- NITOPHYLLUM ODONTOPHORUM Howe & Taylor.—*Hassler*: Dredged off Cabo Frio.
- NITOPHYLLUM UNCINATUM (Turn.) J. Ag.—Plate XXXIX.—*Hassler*: Dredged off Cabo Frio. This material offers much similarity to specimens of *N. uncinatum* from England, California and Japan, which species has been segregated from *N. laceratum*. Uncinate tips are frequent on this material, and in habit, lack of veins, cell size and type of thallus margin it seems much nearer these specimens of *N. uncinatum* than any of *N. laceratum* available.
- ACANTHOPHORA MUSCOIDES (L.) Bory.—*Hassler*: Scarahy and harbor, Rio Janeiro. *Schmitt*: Ilha Paqueta; Villa Bella, Ilha São Sebastião.
- BRYOTHAMNION SEAFORTHII (Turn.) Kg.—*Hassler*: Dredged off the Abrolhos Ids. Det. M. A. Howe.
- BRYOTHAMNION TRIQUETRUM (Gmel.) Howe.—*Albatross*: Dredged off Pernambuco in 20 fathoms.
- CHONDRIA FLORIDANA (Collins) Howe.—*Albatross*: Dredged off Pernambuco in 20 fathoms.
- DASYA SERTULARIOIDES Howe & Taylor.—*Hassler*: Dredged off the Abrolhos Ids.
- LAURENCIA LATA Howe & Taylor.—*Hassler*: Dredged, Cabo Frio.
- LAURENCIA PAPILLOSA (Forsk.) Grev.—*Hassler*: Scarahy. *Schmitt*: Villa Bella, Ilha São Sebastião.
- GRIFFITHSIA RADICANS Kg.—*Schmitt*: Villa Bella, Ilha São Sebastião.
- SPYRIDIA ACULEATA (Schimp.) Kg.—*Hassler*: Harbor of Rio Janeiro. A fragment only, but the ultimate ramuli with the characteristic structure.
- SPYRIDIA FILAMENTOSA (Wulf.) Harv.—*Hassler*: Dredged near the Abrolhos Ids. *Schmitt*: Ilha Paqueta.
- HALYMENIA FLORESIA (Clem.) J. Ag.—*Hassler*: Dredged near the Abrolhos Ids.
- HALYMENIA INTEGRALIS Howe & Taylor.—*Hassler*: Dredged, Cabo Frio.
- HALYMENIA ROSEA Howe & Taylor.—*Hassler*: Dredged, Cabo Frio.
- HALYMENIA VINACEA Howe & Taylor.—*Hassler*: Dredged, Cabo Frio.
- PLATOMA TENUIS Howe & Taylor.—*Hassler*: Dredged, Cabo Frio.

- AMPHIROA BRASILIANA Decne.—*Hassler*: Harbor of Rio Janeiro. *Schmitt*: Pedra de Itapuca, Nictheroy. Det. M. A. Howe.
 AMPHIROA FRAGILISSIMA (L.) Lamx.—*Schmitt*: Pedra de Itapuca, Nictheroy.
 JANIA ADHAERENS Lamx.—*Hassler*: Scarahy.
 JANIA CAPILLACEA Harv.—*Schmitt*: Pedra de Itapuca, Nictheroy.

DISCUSSION

While the amount of material from Brazil was not as large as from some of the other districts visited by the "Hassler," the "Albatross," and Dr. Schmitt, it is of interest in assisting to confirm and extend previous reports of Brazilian algae. Altogether 85 algae are listed here from these collections, and no attempt was made to search for minute epi- and endophytes. In conjunction with the study of these materials it was necessary to compile a complete list of Brazilian algae from various sources and to give general consideration to the character of the marine flora. The results of this compilation and analysis have been brought to a comparatively orderly state and in conjunction with the records of this present report are to be made the subject of a separate paper to appear elsewhere. The algae appearing in these collections which probably represent additions to the recorded flora of the territory are: *Trichodesmium Thiebautii* Gom., *Caulerpa Ashmeadii* Harv., *C. lanuginosa* J. Ag., *C. racemosa laetevirens* (Mont.) W.-v. B., ?*Codium Pilgeri* Schmidt, *Dasycladus vermicularis* (Scop.) Krasser, *Dictyosphaeria favulosa* (C. Ag.) Decne., *Enteromorpha flexuosa* (Wulf.) J. Ag., *Rhiphilia tomentosa* Kg., *Udotea cyathiformis* Decne., *Valonia ventricosa* J. Ag., *Dilophus alternans* J. Ag., *Sporochnus Bolleanus* Mont., *S. pedunculatus* (Huds.) C. Ag., *Chondria floridana* (Collins) Howe, *Laurencia lata* Howe & Taylor, *Chrysomenia planifrons* (Melv.) J. Ag., *C. pyriformis* Børg., *Rhodophyllis gracilarioides* Howe & Taylor, *Gracilaria mamillaris* (Mont.) Howe, *Faucheia Hassleri* Howe & Taylor, *Nitophyllum odontophorum* Howe & Taylor, *Nitophyllum uncinatum* (Turn.) J. Ag., *Dasya sertularioides* Howe & Taylor, *Spyridia aculeata* (Schimp.) Kg., ?*Eucheuma Gelidium* J. Ag., *Halymenia integra* Howe & Taylor, *H. rosea* Howe & Taylor, *H. vinacea* Howe & Taylor, *Platoma tenuis* Howe & Taylor. In addition, many extensions of range are involved.

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DESCRIPTION OF PLATE XXXIX

Nilophyllum uncinatum. Several individuals. The specimens are by no means as large and handsome as some from other places seen by the writer. However, this is in part due to the fragmentary nature of the specimens and the comparatively inexpert fashion in which they were mounted. The uncinata branchlets have in part been marked with arrows. Sometimes they were flat, sometimes apparently subcylindrical, especially toward the tip. $\times 3/4$.



TAYLOR: BRAZILIAN ALGAE

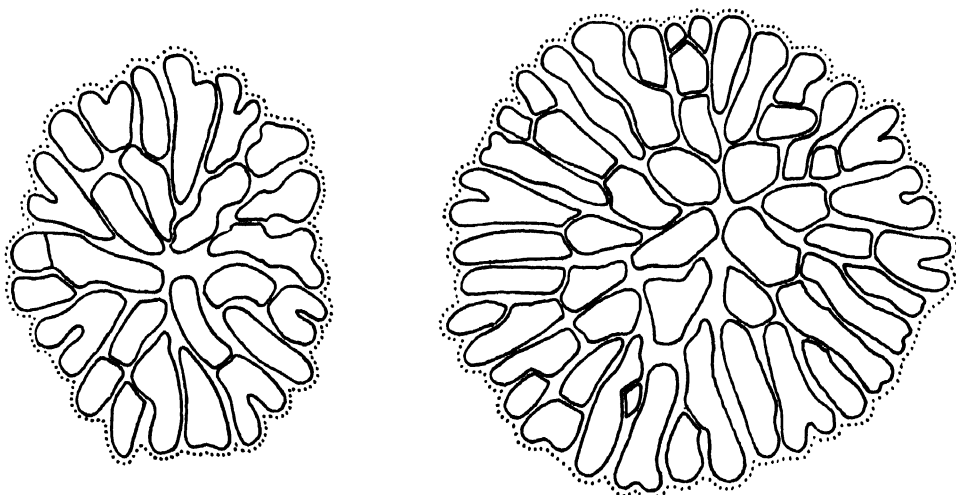
NOTE ON MARINE ALGAE FROM SAO PAULO, BRAZIL

WM. RANDOLPH TAYLOR

(Received for publication February 22, 1930)

After the preceding paper had been completed, a small collection of marine algae assembled in Brazil by L. B. Smith was sent to the writer by the kindness of Dr. C. W. Dodge, with permission to study and report on the contents. Some items (*) are new records, and very interesting. The following list includes the entire collection:

- *LYNGBYA CONFEROIDES C. Ag. ex Gom.—Conceição de Itanhaen.
- ENTEROMORPHA COMPRESSA (L.) Grev.—Guarujá; Conceição de Itanhaen.
- ULVA FASCIATA (L.) Grev.—Guarujá; Conceição de Itanhaen; M. Jundiahy, São Vicente.
- NEUROCARPUS DELICATULUS (Lamx.) O. Ktze.—Guarujá, on *Sargassum*.
- SARGASSUM CYMOSUM J. Ag.—Guarujá.
- *ERYTHROCLADIA SUBINTEGRA Rosenv.—Conceição de Itanhaen; M. Jundiahy, São Vicente. On *Chaetomorpha* (text fig. 1).



TEXT FIG. 1. Two small colonies of *Erythrocladia subintegra*. When larger the colonies often became elongated, extending over more than one segment of the host. $\times 820$.

- PORPHYRA sp.—Guarujá.
- AMPHIBIA TENELLA (Vahl.) Ktze.—M. Jundiahy, São Vicente.
- *BRYOCLADIA THYRSIGERA (J. Ag.) Schmitz.—M. Jundiahy, São Vicente.
- CENTROCERAS CLAVULATUM (C. Ag.) Mont.—Guarujá; Conceição de Itanhaen; M. Jundiahy, São Vicente.
- AMPHIROA BRASILIANA Dcne.—Guarujá.

A SIMPLIFIED SILICA GEL

J. FRANKLIN STYER

(Received for publication February 25, 1930)

In connection with nutrition experiments on the cultivated mushroom a silica base plate has been devised which can be adapted to general use in mycology and plant physiology. The use of silica gel has been restricted to the study of cellulose-destroying and soil organisms by the supposed difficulty of preparation. This method is neither complicated nor expensive, and saves much of the time and space taken by the older preparations.

The Omeliansky silica gel preparation as described by Smith (2) consists of the addition of sodium silicate to hydrochloric acid, both of such low specific gravity that gel formation is delayed until the sodium chlorid can be dialyzed through a collodion sack and the nutrient added. The Winoogradsky method (4) and its improvement by Waksman and Carey (3) use stronger silicate and acid, and the gel sets almost at once. It must then be dialyzed in tap or distilled water for one or two days, and the nutrients must be added by diffusion from the surface.

The method of Doryland (1) makes the obvious improvement of substituting potassium silicate for sodium silicate, and phosphoric acid for hydrochloric acid. The salts and ions of potassium and phosphate do not need to be completely dialyzed from the gel, as they are nutrients. The other nutrients are added to the acid solution before it is neutralized with the silicate.

The writer used features of all these methods to reduce the time and simplify the technique of gel making. An analyzed silicate solution was used in place of solid silicate, which is slow to dissolve and unknown in potash content. Phosphoric acid was used, to produce a nutrient gel. The ingredients were diluted so that they could be handled and poured in quantity before setting. The concentration of potassium and phosphate was adjusted by partial dialyzing when necessary. Nutrients were added to the acid solution or in powder form to the surface of the gel. No care was used to make the gel sterile, as autoclaving was found to be perfectly feasible.

The following directions will serve as a basis for making this medium for a variety of purposes. An analyzed clear solution of potassium silicate is obtained, preferably with a molecular ratio of K_2O to SiO_2 of 1 to 3.5 or lower, so that the least amount of potassium salts will be formed. The specific gravity is immaterial. Such a solution will usually contain about 20 percent SiO_2 . As a trial, 20 cc. of the silicate are diluted to 100 cc., and neutralized to litmus with about 0.2 M H_3PO_4 , and successive portions

diluted with increasing amounts of water. The portions are set aside to gel, and that which gels in about 15 minutes is taken as the limit of safe dilution; this limit is usually about 1.2 percent SiO_2 . The concentration of potassium and phosphate is now calculated and the nutrient solution planned. The nutrient gel is made by adding the extra nutrients to the acid solution, or by withholding them to be added in powder form to the surface of the gel, as preferred. The mixture can be made by the liter and poured into plates or tubes without hurry.

The balancing of the nutrients requires some care. A gel made as described above will be about 0.1 *M* with respect to potassium phosphates. There are many cases in which this concentration will be too high. The procedure in such cases is to add all the other nutrients in correspondingly high concentrations and then dialyze in a large pan of water until the correct concentration is reached. For example, when a solution 0.05 *M* with respect to phosphate is suitable for an organism, the gel is made up double strength of all nutrients and then placed in water for about three hours. The speed with which this reduction takes place must be determined by experiment. It is suggested that more accurate results will be obtained by pouring a small measured amount of distilled water on the gel and leaving it there to take up a maximum amount of dialyzable materials before removing it. This procedure is usually satisfactory and is always quicker than complete dialyzing and subsequent addition. The amount of acid can be adjusted to give exactly the pH value desired, although the gel sets more quickly near pH 6.0 to 7.0. The reaction can also be adjusted by adding acid or alkali before autoclaving.

The nutrients added in the form of powders, if soluble, are distributed through the gel during autoclaving, or on standing a few hours. Insoluble materials may readily be spread on the surface with a salt shaker. Autoclaving the plates is quite possible and no change occurs in the gel.

The finished gel is as clear as agar and does not dry out any faster. Its chief advantage lies in its freedom from organic materials. Green plants may be grown on it with a minimum of saprophytic contamination, and media for the study of saprophytes may be made of it in which the organic content is known and may be changed at will.

It is difficult to obtain potassium silicate of a good grade with analysis. The samples used in this work are being supplied by the Philadelphia Quartz Co., and are a part of their regular stock. Dr. William Stericker of that firm has assisted the writer with very helpful suggestions.

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CRUCIFER CARPELS

ARTHUR J. EAMES AND CARL L. WILSON

(Received for publication March 1, 1930)

The recent publication by the authors (1) of certain facts and theories concerning the nature of the ovary in the crucifers has called forth in reply a paper by Miss Saunders (4), in which certain major and minor points are dealt with controversially. It is evident that the differences of opinion have resulted in large part from a misunderstanding of the position of the authors in respect to the carpel polymorphism theory; from a lack of clearness of definition of the "solid" and the "semi-solid" carpel; and from a misinterpretation of the anatomy of the carpel as understood under the monomorphic theory. It seems necessary that viewpoints and anatomical bases of interpretation be made more clear, and the points of difference critically examined.

The authors, in attempting to show in what way the modified carpels of the crucifers suggest in their structure a peculiarity in carpel type, did not make sufficiently clear their opposition to the polymorphism theory, and their position was interpreted as supporting the polymorphism theory so far as the crucifers are concerned. They desire, first, to state emphatically their adherence to the long-established theory of the nature of carpels—to what Miss Saunders calls the monomorphic theory. They deny the existence of any fundamental difference in the carpels of angiosperms, even in the Cruciferae, maintaining that all carpels are strictly homologous. Anatomical structure, in their opinion, firmly supports the monomorphic theory.

In the publication of their paper they desired not only to present facts and theories concerning the crucifer ovary additional to those already published, but to support Miss Saunders in her position that this ovary is made up of four carpels, in opposition to the current opinion that there are but two carpels present. They showed what was, in their opinion, evidence of the presence of four carpels still more convincing than that which had been presented by Miss Saunders, and showed, at the same time, that the "solid" carpels were typical, true carpels anatomically. They believed that this aided in the establishment of the formula $G = 4$ for the Cruciferae, but they were obliged to interpret the ovary of other families and other features of crucifer ovary structure in ways at variance with the polymorphism theory.

The present writers have at no time been able to abstract from Miss Saunders' long, complex, and obscure presentations of her theory any clear idea of what the "solid" and "semi-solid" carpel are conceived to be from the standpoint of comparative morphology. The term polymorphism

implies the existence morphologically of more than one type of carpel. How fundamental in the opinion of Miss Saunders is the difference in type, it is difficult to ascertain. The first paper (3) on carpel polymorphism describes "carpels of different structural types, fulfilling different functions"; and adds that "throughout the whole range of flowering plants we meet with reduction in number and 'consolidation' of the members of the gynoecium, the latter process having resulted in the production of certain definite carpel forms and having been accompanied by a redistribution of the carpellary functions." The "hollow or valve type" is described as "probably the most primitive." It is evident that the polymorphism does not extend back to the origin of the angiosperms; the modifications have appeared during the development of the group. There has been modification of the earlier type and "consolidation of members of the gynoecium." Since the valve carpel is "probably the most primitive," the solid carpel is a "consolidated" one, a reduced one. And this reduced carpel often bears the ovules,—is fertile (except when it is sterile).

There seems to be no criterion by which one, other than the proponent of the theory, can judge the nature of the carpels in a given case. The solid carpel is either fertile or sterile; it has a vascular supply of one bundle or of two bundles, the two side by side tangentially or side by side radially; the "one bundle" of the solid carpel has been formed ontogenetically by the fusion of a ring of bundles through the development of "mechanical tissue" between them (truly an extraordinary morphological origin), or is a simple vascular strand. It appears to the authors that the solid carpel may have any anatomical structure, or any form whatsoever, and any function which suits the occasion. And when, in spite of all this leeway, valve and solid carpels cannot be made to explain the structural condition, because of some peculiarity of secondary venation, the convenient and flexible semi-solid carpel can always be adjusted to the situation.

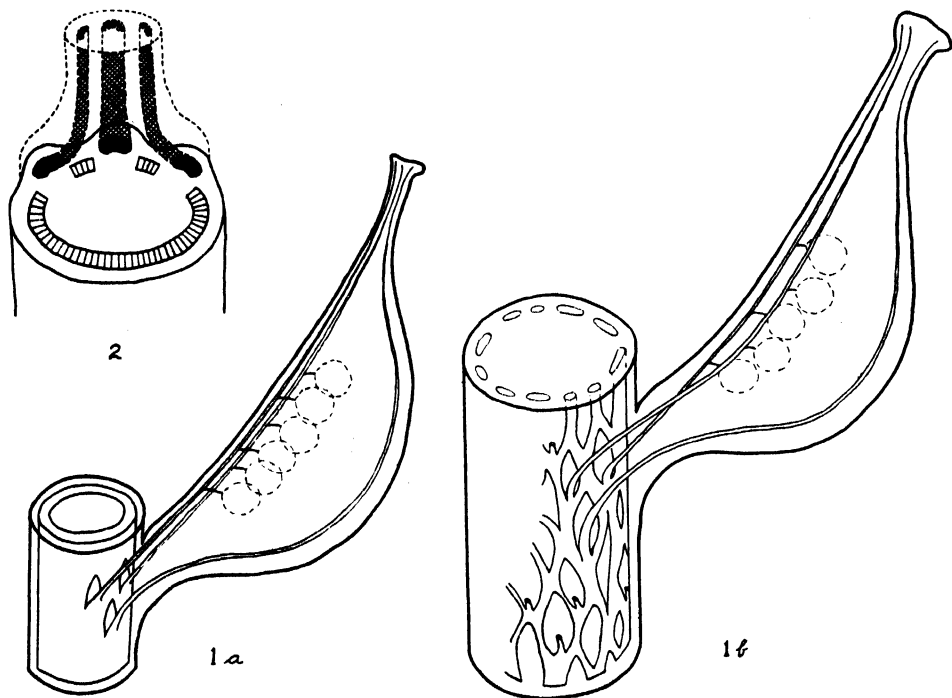
Since the polymorphism theory owes its origin to the structure of the crucifer ovary, it is necessary, in the first place, that the structure of this organ be reconsidered.

The nature of the crucifer ovary has long aroused controversy, and all students have believed it to have peculiar structure. According to Miss Saunders there are present carpels of distinct types, varying greatly in form and function. This condition has appeared to her to be of the utmost importance to an understanding of the morphology of ovaries in general. To the present writers the peculiar carpels of this family represent merely an extreme modification of the normal carpel, developed in a small group of families within the Rhoeadales and probably not found elsewhere.

The theory of polymorphism, established upon this peculiarity of structure in the crucifers, has been extended by its proponent to many other angiosperm families; in fact, polymorphism is claimed to exist probably

in all others. In these other families—outside allied families within the Rhoeadales—there exists, in the present writers' opinion, no similar or even comparable condition. That is, there does exist in a part of the Rhoeadales a quasi-polymorphism (lest there be misunderstanding, a *false* polymorphism), but similar conditions do not exist in other orders. In the writers' opinion a morphological peculiarity or modification restricted to a small group of families has been, in an exaggerated form, "read into" the structure of many other families, in the elaboration of the polymorphism theory.

The writers' position in regard to the structure of the crucifer ovary can only be made clear by an explanation, in brief, of their understanding of typical carpel anatomy. This has already been presented in the earlier paper, but must be repeated here. The carpel is morphologically a lateral



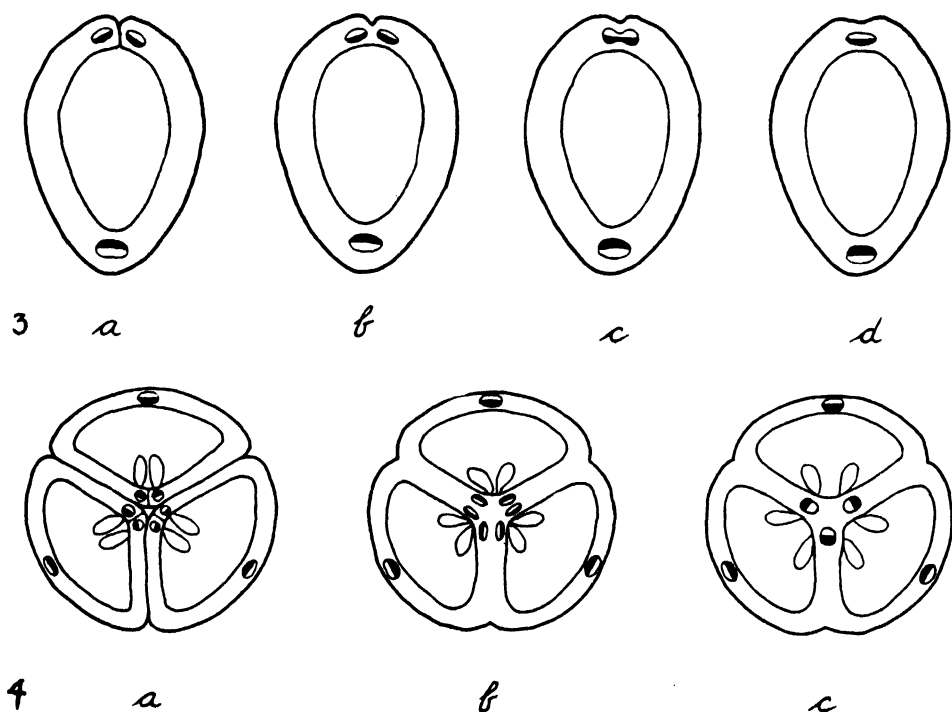
TEXT FIG. 1. Diagrams showing the vascular supply of a typical carpel; the three separate traces derived, *a*, from an unbroken cylinder, *b*, from a dictyostelic cylinder.

TEXT FIG. 2. Diagram showing origin of traces to a typical leaf,—three bundles leaving three gaps. (After Sinnott and Bailey.)

appendage, a leaf or leaf-like structure, folded upward along the midrib, the ventral surface being enclosed by the bringing together of the margins. The margins bear ventrally the placentae. The follicle is commonly looked upon as the type of such a carpel representing a primitive condition. Its venation is as follows: three bundles leave the receptacular stele as traces, one median and two lateral, the lateral traces *departing separately* and

slightly higher up on the receptacle than the median (text fig. 1). This is exactly the trace situation in typical angiosperm leaves (text fig. 2). These three traces pass separately into the carpel base and upward through the carpel, the median trace becoming the dorsal or midrib bundle, the lateral traces becoming bundles running along the carpel margins and called the marginal bundles or, because of their position, the ventral bundles. The approximation of the margins brings the ventrals close together and they are often found fused laterally to a greater or less extent (text fig. 3), though in their origin at the stele of the receptacle entirely separate and even far apart on the circumference of the stele.

In the case of syncarpous ovaries, the carpels are fused to one another laterally, either in the closed state, as in *Lilium*, when their sides are in



TEXT FIG. 3. Diagrammatic cross-sections of typical carpels (follicle type), showing various degrees of approximation and fusion of ventral bundles. In each case there are two ventral traces, derived separately, and a dorsal trace. TEXT FIG. 4. Diagrammatic cross-sections of syncarpous ovaries, showing various degrees of approximation and fusion of the ventral bundles. These bundles fuse in pairs, a pair consisting of bundles from two adjacent carpels.

contact, or in the open state, as in *Viola*, when their margins touch. Here, too, approximation of ventral bundles may bring about fusion, complete or partial, but the bundles which are brought together are the marginals of different carpels (text fig. 4). Fusion occurs as freely as though both

bundles belonged to the same carpel. The fusion of bundles belonging to separate organs is not extreme, of course, for fusion of bundles even of different types of organs occurs commonly when they lie close together; for example, the bundle of an epipetalous stamen is fused to the bundle of the petal which bears it.

On the basis of the above understanding of fundamental ovary anatomy in the angiosperms, the crucifer ovary readily falls into line. Two whorls of two carpels occur, one as distinctly above the other as two of the sepals in this family are above the other two. Each of the four carpels receives the normal three traces from the stele of the receptacle in the normal manner.¹ It is in the structure of the carpels themselves that the crucifers stand apart from other angiosperms. Typically in angiosperms where more than one whorl of carpels enters into the formation of a syncarpous ovary, the whorls stand so closely that there is no evidence (other than anatomical) that alternate carpels are at higher levels. But in the crucifers the difference in level is marked. And associated with this difference, and doubtless correlated therewith, is a great difference in form and function in the two whorls. The two lower carpels are open, and clasp, as it were, the upper two, more or less nearly enclosing them. In some genera the enclosure is all but complete. It is believed by the writers that the development of this peculiar ovary has been along the following lines indicated in text figure 5. With (primitively) four open, marginally fertile carpels (text fig. 5, *a*), the evolutionary tendencies toward closure of the carpels and cohesion among the carpels have found expression simultaneously. The same tendencies have been expressed in many other families, but in this case, the resulting syncarpous structure is unusual; and the unusual condition is, in some measure at least, due to the greater distance between the two whorls. This difference in level has prevented a fusion in which all carpels are affected similarly or closely similarly as in most syncarpous families. The upper two carpels have become closed; the lower two have become appressed to the upper, and fused thereto (text fig. 5, *b*, *c*, *d*). The relative positions of the whorls, with other factors, have prevented the closing of the lower whorl.

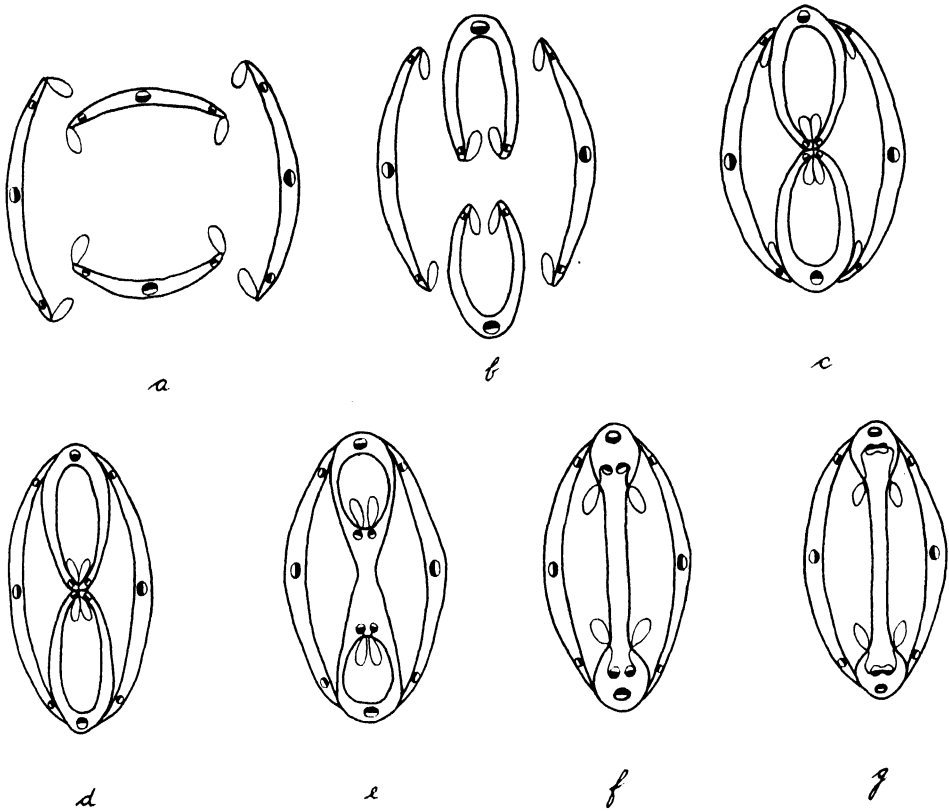
The upper whorl, nearly enclosed by the lower (text fig. 5, *c*, *d*), has become reduced, as might be expected of organs, normally external, which become essentially internal. Reduction has been by contraction, contraction so great that the loculus has disappeared (text fig. 5, *d*, *e*, *f*). However, the vascular system remains essentially the same; *the position, size, number, and orientation of the bundles is still as in a typical carpel* (text fig. 5, *f*, *g*). These facts are of the greatest importance.

In spite of the reduction, the carpels remain fertile, however, and *the ovules are attached to the ventral bundles* (text fig. 5, *f*) of these contracted

¹ Miss Saunders states that this is not the case, but misinterprets the derivation of the last traces from the flower stele (see p. 641).

carpels. The outer carpels, which serve merely as protecting structures, have lost their fertility, but retain the bundle supply of a typical carpel. The retention of fertility by the inner carpels while contraction has been so great as to obliterate the loculus, has necessarily involved the extrusion of the ovules from the original loculus (text fig. 5, *f*). The ovules therefore lie outside of the closed carpel which bears them and have a position in a loculus limited largely by a different carpel (text fig. 5, *f*, *g*).

The writers maintain that there is in the vascular supply of crucifer



TEXT FIG. 5. Diagrams showing the authors' opinion of the method of development of the crucifer ovary: *a*, two whorls of two open fertile carpels; *b*, the inner whorl partly closed; *c*, the four carpels brought together, the inner two closed, the outer two appressed, still open, to the inner two; *d*, the outer two have become sterile; *e*, the inner two are contracting, the loculus becoming small, the ventral bundles retreating from their marginal positions, following the ovules; *f*, the loculi of the inner carpels obliterated, the ovules pushed outside the carpels, but still attached in a normal way to the ventral bundles; *g*, the ventral bundles of the inner carpels fused.

ovaries nothing extraordinary. The skeleton is that which in number, position, and orientation of its parts is to be expected in two whorls of two carpels each, one above the other. The writers further maintain that there is nothing here in carpel structure which can serve as a basis for poly-

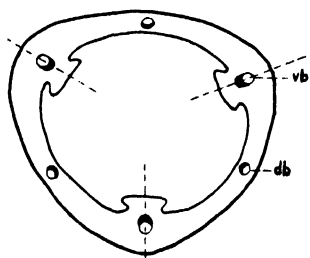
morphism. They grant that the position of the ovules—extruded from the carpels which bear them into external regions—is unusual. They object, however, to the statement of Miss Saunders that such ovules are “*endogenous*” in origin. This term leads to the ready inference that the present writers maintain that the ovules in the crucifers are developed deep within the tissues of the carpel itself and then burst out through the epidermis. This impression is increased by Miss Saunders’ incorrect statement (4, p. 123) that in their view “the ovules of the Cruciferae develop in a manner profoundly different from that in which they arise in other families.” The present writers maintain that crucifer ovules are borne as ‘exogenously’ as any angiosperm ovules, but that they have become extruded from the locus. This extrusion has occurred directly through the carpel wall, but *phylogenetically, not ontogenetically*. Therefore direct histological and ontogenetic evidence is not to be expected, although it has not entirely disappeared (see below). Such direct extrusion of ovules, ontogenetically, through the carpel wall occurs in *Caulophyllum thalictroides*. Here is in ontogeny what the writers maintain has occurred in phylogeny in the crucifers.

The statements of the authors concerning the trace conditions in carpels are misrepresented. Miss Saunders (4, p. 127) quotes the authors (1, p. 253) correctly as follows: “Normally, in the majority of angiosperm families, a carpel receives three traces—a dorsal, or midrib bundle, and two ventral or marginal bundles.” She follows the quotation with these words: “The reader might take this to be a statement of fact, but the statement holds *only* if the theory of polymorphism is rejected altogether.” The authors maintain that this is a statement of fact, and that it was intended that the reader should take it as such. When making the statement they held, as they still do, to the monomorphic theory. It is apparent that the proponent of the polymorphism theory believes that the presentation of the new theory has been sufficient for its general acceptance. This view is evident elsewhere through the papers on polymorphism, where it appears that the fact that any student might still adhere to the monomorphic theory is so unlikely that consideration of such a viewpoint may usually be neglected. The authors have seen no published paper in which the theory has been accepted.

Somewhat further on, Miss Saunders states that “the authors appear to withdraw somewhat from their earlier position, for they state that ‘the number [of traces] in a few families is doubtless more [than 3], and in the case of reduced pistils the three bundles are clearly reduced to one.’ So that their generalization thus modified appears to say only that the number of traces to the carpel is 3 when it is not some other number.” This statement is similar to others in the paper where the authors’ words are distorted or misinterpreted. The authors’ first statement, the general one, was: “Normally, in the majority of angiosperm families, a carpel

receives three traces." The second statement was that "the number [of traces] in a few families is doubtless more [than 3], and in the case of reduced pistils the three bundles are clearly reduced to one." The authors submit that therein they do not "withdraw somewhat from their earlier position"; that their "generalization thus modified" does not "appear to say only that the number of traces to the carpel is 3 when it is not some other number." The second statement in no way conflicts with the first.

The statement of the authors that the ovules of the crucifers "lie outside the carpel that bears them" is critically discussed by Miss Saunders, but the criticism is written as though the authors subscribed to the polymorphism theory. The fact that the authors have stated in several places that they consider the so-called solid carpels of the crucifers to be solid *only* in the sense that the loculus has been eliminated by contraction is overlooked; yet Miss Saunders herself discusses these carpels as in the authors' opinion "quasi-solid." After a discussion of the fact that ovules are never borne *inside* a carpel—in the sense of within the tissues of the carpel—Miss Saunders asks: "Is it not obvious that this condition [of ovules lying outside the carpel which bears them] is to be found in all polymorous, unilocular ovaries?" Certainly not, except from the viewpoint of polymorphism. For in such an ovary² (text fig. 6), none of the carpels is closed, and ovules in such cases can lie neither inside nor outside of the carpels which bear them. The criticism overlooks completely the main and repeated con-



TEXT FIG. 6. Cross-section of ovary of *Viola* showing the vascular supply, placentae, and carpel limits.

tention of the authors that the fertile carpels of the Crucifers are closed, completely, as in a typical follicle. The closed carpels have become contracted until their loculi are lost. The bundles, however, are the same in number, relative position, and orientation (as Miss Saunders herself states); this constitutes proof of the contraction and consolidation which she claims has occurred, but which she is unwilling to consider in detail because of

² It is stated that the authors "apparently believe" that this ovary has three carpels. The figure, which is here repeated, definitely shows three carpels, "showing the vascular supply and carpel limits." The authors' statements are in other places similarly distorted in ways that seem to weaken them.

the obvious difficulty of the change of position of the ovules from the loculus to the "outside" of the carpel. Miss Saunders' own arguments for the derivation of the solid carpel should have led her logically to just this conclusion.

Further, and of critical importance, *the attachment of the ovules is to the innermost,—the inverted—two bundles of the ring of bundles in a solid carpel, the very two (the marginal) bundles to which the ovules would be attached if the loculus were still present.* Clearly, if contraction has occurred until the loculus has disappeared (and Miss Saunders has figured and described such in *Triglochin*) the ovules must have been lost. But, on the polymorphism theory they are found on the outside of the carpel, on either side of the inner face. The polymorphism theory demands that the ovules be lost, and that the now sterile solidified carpel become fertile again by the development of ovules anew on its outer surface! This is an extraordinary conclusion. Yet the theory presented by the authors that the contraction of the carpels and loss of the loculus has brought about extrusion of ovules through the walls is looked upon by Miss Saunders as still more extraordinary; and it is claimed that the authors argue for such a condition from an *a priori* base, and that there is no such base. The authors submit that there is, in the vascular structure of these carpels—*the arrangement and orientation of the bundles and the point of attachment of the ovule traces*—the strongest possible *a priori* argument for the fact of ovular extrusion.

The extrusion of ovules is of course remarkable (though not unknown, e.g. *Caulophyllum*) and the authors themselves at first accepted this explanation only because obliged to do so from the compelling evidence of the vascular supply. They sought histological and ontogenetic evidence, and found sufficient to reinforce their opinions.

Miss Saunders argues that if the ovules had been extruded as claimed, there would be histological evidence thereof in the superficial layers of the septum, i.e., the 'break' would be evident in the epidermal tissues of the fertile carpels, and states that her studies of young buds show that such evidence does not exist. But, even if the extrusion were ontogenetic, this evidence might be lacking. Miss Saunders herself describes a case in point. In the ontogeny of the ovary the septum is formed, as is well known, by the union of two lobes which develop from the young median carpels. These projections each have a well-defined epidermis; yet in the region where the lobes touch and fuse, there is soon no histological evidence of the fusion. The single structure shows no proof of its double origin.

However, the authors did not, and do not, claim that the extrusion takes place ontogenetically. Believing, during their studies, that extrusion might perhaps so occur, they searched most carefully, and presented all the histological evidence they found—that of stomates on the funicle, of 'shoulders' on the chalaza, and of ensheathment of the funicle. The statements of the authors that they found no other histological evidence is in

precise agreement with that of Miss Saunders. The strictly histological evidence of extrusion is weak, it is granted, and alone would be of little value, but undeniable evidence exists in the anatomy,—in the distribution, orientation, and attachment of the vascular bundles. The histological evidence provides merely additional support for the theory.

The authors are told that the point upon which they lay great stress, the inverse orientation of marginal bundles, is worthless, and that their "attitude springs from a misconception regarding their origin, for as *lateral* veins their arrangement is natural and what is to be expected. . . . In the solid carpel . . . the veins all face the center" (4, p. 129). The authors agree that in a folded carpel the veins all face the center. This is the critical point.

In the first place it is not clear what is meant by the statement that what the authors call marginal veins are *lateral*. In a sense, of course, all veins except the midrib are lateral; or it may be implied that the marginal veins are not true traces derived from the stele, but are branches of the midrib, or dorsal, trace. The authors maintain that the marginal veins are true traces, and are wholly independent in their origin. But whatever the nature of the bundles, marginal or lateral, inversion is a matter related to the nature of the ovary. In an apocarpous pistil certainly all bundles, whether traces or branches of the traces, face the center, as they must if the pistil represents an upfolded leaf, and no bundles can then be said to be inverted. But with the union of carpels in syncarpy the marginal bundles become inverted as compared with the dorsal bundle and its branches (text fig. 4); that is, from the standpoint of the ovary the marginal bundles have xylem toward the outside rather than phloem. They are therefore considered inverted, and the inversion is proof of their nature, as Van Tieghem and others long ago said. Now the crucifer ovary, whatever its carpellary make-up, is syncarpous. The presence of inverted bundles is important. And when those bundles are the ones to which the ovules are attached, this fact is of the utmost importance; it determines the position of carpels.

If these bundles were considered laterals in the sense of not true marginal traces, it should have been explained how they could bear ovules, for nowhere in carpels (unless under "scattered" placentation) are ovules attached to branches of the midrib. (The situation in achenes is wholly different and cannot be gone into here.)

The authors have found in Miss Saunders' papers no explanation, from the standpoint of polymorphism, of this critically important point that the *ovule traces are attached to these inner inverted bundles*. In fact, ovule attachment in the crucifers is most inadequately discussed by Miss Saunders, and in no case is figured except in longitudinal views of fruits where it is not possible to show to what part of the complex vascular system of the fertile carpel the ovule is attached. No explanation is made of this lack; the

reason is doubtless that the actual attachment of the bundle was not often seen, for Miss Saunders speaks of sections of flower buds and flowers. An acquaintance with flower anatomy suffices to show that in many families the ovule traces are not connected with the mother strands until after flowering is past. In the crucifers ovule trace attachment is very rarely made until after the petals have fallen.

Objection is raised to the description by the authors of the fertile carpels of the crucifers as "reduced and abortive"; it is stated that organs that are "abortive" could not possibly be fertile. Regardless of the lack of the really necessary definition of this term in either paper, it is claimed in each case that the solid carpel is a reduced structure, formed by contraction or "solidification." Some at least of these reduced carpels—those of the crucifers and allied families—are fertile. And it is remarkable that Miss Saunders should object to the claim of the writers that a reduced carpel can remain fertile, when the "type" solid carpels,—those first described by her,—are fertile. Parkin (2) objected to Miss Saunders' derivation of the solid carpel, stating that it is difficult to understand how a carpel can retain its fertility during reduction, and was taken vigorously to task therefor; yet Miss Saunders now takes the same position in regard to the statement of the authors which she severely criticized Parkin for assuming in regard to her theory.

In the criticism of the authors' statement that the ovules are attached to the quasi-solid carpels and have derived their position by phylogenetic extrusion through the carpellary wall, Miss Saunders states that "they [the authors] cite only a single case, *Lepidium virginicum*." This is a serious misrepresentation of the facts presented in their paper, as an examination will show. *Lepidium virginicum* is the species used for text figure 1, which is obviously presented to illustrate the crucifer ovary, just as *Viola* and *Trillium* are used in the same plate to illustrate other ovary types. Further, their text figure 6 is another general illustration presented to show this very fact; and its legend "Diagram of part of a crucifer ovary showing relation of ovule to septum . . ." should make clear the fact that the writers believe this to be the condition in the family. Miss Saunders notes that "they state their belief that the condition which they describe as existing in this species [*Lepidium virginicum*] is present in other genera of the family." Their published statements are certainly to this effect: "in many cases" (p. 262); "among the many species studied by the authors"; and "*Brassica nigra* shows this [freedom of the funicle within the septum as indicating origin of ovule] more clearly than other species." Apparently a general statement, with citation of examples among species readily obtainable, is insufficient, and space must be given to the listing of genera and species which the writers have studied and are convinced possess the structure claimed for *Brassica nigra* and *Lepidium virginicum*. The list follows:

| | |
|---|--|
| <i>Alyssum saxatile</i> L. | <i>Erysimum cheiranthoides</i> L. |
| <i>Arabis alpina</i> L. | <i>Hesperis matronalis</i> L. |
| <i>Barbarea vulgaris</i> R. Br. | <i>Iberis amara</i> L. |
| <i>Berteroa incana</i> (L.) DC. | <i>I. gibraltarica</i> L. |
| <i>Brassica arvensis</i> (L.) Ktze. | <i>I. umbellata</i> L. |
| <i>B. juncea</i> (L.) Cosson. | <i>Lepidium campestre</i> (L.) R. Br. |
| <i>B. nigra</i> (L.) Koch | <i>L. virginicum</i> L. |
| <i>Camelina microcarpa</i> Andr. | <i>Lunaria annua</i> L. |
| <i>Capsella Bursa-pastoris</i> (L.) Medic. | <i>Mathiola incana</i> R. Br. |
| <i>Cardamine Douglassii</i> (Torr.) | <i>Nasturtium Nasturtium-aquaticum</i> |
| Britton | (L.) Karst. |
| <i>Cheiranthus Cheiri</i> L. | <i>Radicula Armoracia</i> (L.) Robins |
| <i>C. Allionii</i> Hort. | <i>Raphanus Raphanistrum</i> L. |
| <i>Conringia orientalis</i> (L.) Dumort. | <i>R. sativus</i> L. |
| <i>Dentaria laciniata</i> Muhl. | <i>Roripa austriaca</i> |
| <i>Erucastrum gallicum</i> (Willd.) Schultz | <i>Sisymbrium altissimum</i> L. |
| | <i>Thlaspi arvense</i> L. |

The authors have said that all three traces to the carpels—both those to the fertile and those to the sterile carpels—arise *separately* and *individually* from the floral stele. This statement is called “incorrect,”—“the erroneous deduction (of 3 traces to a carpel) is due to the fact that the authors’ observations start at too high a level” (4, p. 128). The authors’ observations were in all cases made from serial sections starting in the pedicel well below the flower. The interpretation of the anatomy of any appendage can of course only be made on a basis of study of the actual *departure* of the trace from the stele; and the authors, students of anatomy and comparative morphology, did not neglect what is clearly most important in the determination of the number and character of the carpels in an ovary, the *point of origin* of each trace.

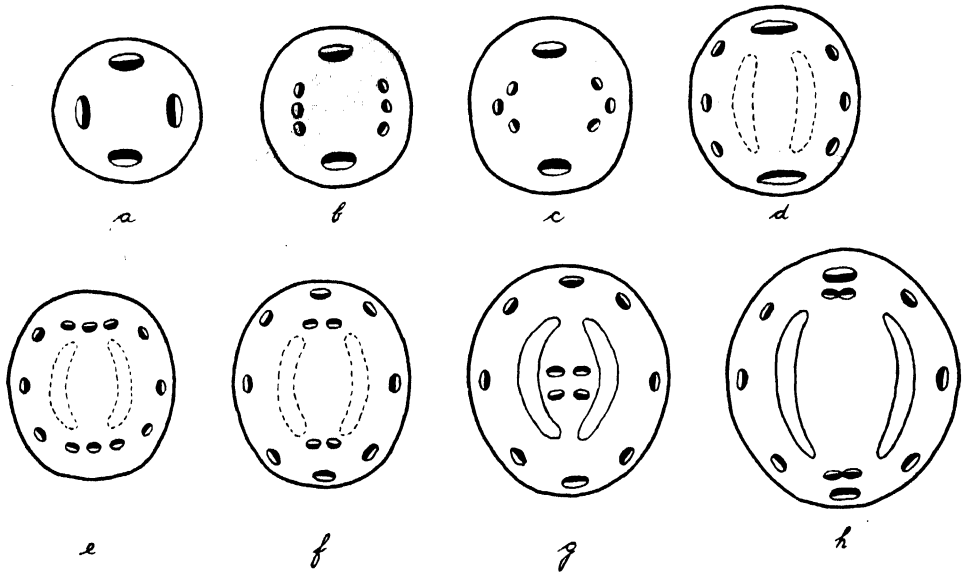
The erroneous deduction has been made by Miss Saunders. In her reply to the authors the statement is made (4, p. 127 and 128) that in those genera in which the single trace to a solid carpel gives off “twin placental strands,”³ or in cases where these cords are unusually massive, . . . first breaks up into a ring of bundles,”⁴ and in those genera where there are not only the four usual “cords,” but four or eight others (to extra whorls of carpels), “only as many cords as carpels enter the gynoeceium, and the secondary veins [those claimed by the present writers to be wholly or in major part the marginal veins] arise as lateral veins from these cords and not directly from the residual vascular cylinder.” It is said that the authors’ statement concerning “the mode of origin of the carpel vascular system in the Cruciferae in general is incorrect, in that it indicates that the pairs of secondary veins

³ The bundle which is in her opinion “the single trace” is a stelar bundle; and the “twin placental strands” are the inverted bundles, or true marginal traces.

⁴ Of which the inverted bundles or marginal traces are a part.

arise directly from the residual vascular cylinder instead of as lateral branches of the midrib cords." The authors maintain that their position is correct, and that Miss Saunders is laboring under the difficulties of a misunderstanding and misinterpretation of flower anatomy in general.

The flower is a determinate branch and in most cases has a stele made up of discrete bundles which are still further split up by the gaps of the traces to the floral organs. Near the top of the receptacle a ring of bundles remains and these bundles—true stelar bundles—all pass out as traces to the uppermost organ or organs, as the case may be. The mistake made in



TEXT FIG. 7. Diagrammatic cross-sections through the base of a typical crucifer ovary, showing the origin of the traces to the carpels: *a*, four bundles constitute the receptacular stele above the stamens; *b*, the dorsal traces of the lateral carpels are freed; *c*, they pass out, leaving six receptacular bundles; *d*, the four lateral bundles pass out as the ventral traces of the lateral carpels, and the loculi of these carpels appear; *e*, *f*, the dorsal traces of the upper carpels are freed, leaving four receptacular bundles; *g*, the remaining receptacular bundles pass inward as the ventral traces of the upper carpels, becoming inverted as they do so; *h*, these ventrals retreat to a position near the dorsals (no loculi being present in these reduced carpels).

interpretation in the case of the crucifer flower is in calling the four bundles remaining after the stamen traces have passed out, four carpel *traces*. These bundles are not carpel traces, but represent the receptacular stele, which at this level consists of four bundles (text fig. 7, *a*). From two of these, strands are given off medianly, the dorsal traces of the sterile carpels, and gaps are left (fig. 7, *b*, *c*). There are now six bundles in the floral stele (eight in the entire receptacle). The four smaller stelar bundles now pass out as the ventral traces of the sterile carpels, leaving in the stele itself only the other two large bundles (text fig. 7, *d*). It is obvious that the two

sterile carpels are much lower than the two fertile ones because the loculi of these carpels appear even before the dorsal traces of the fertile carpels depart from the two remaining stelar bundles. The dorsal traces are next freed from the two other bundles (text fig. 7, *e*) and pass out, followed by the four other bundles (the ventrals of the fertile carpel), which swing inward (text fig. 7, *f*) as do ventrals in uppermost follicular ovaries in any family. (The ventrals then swing back toward the outside (text fig. 7, *g*) owing to the contraction of the loculus.)

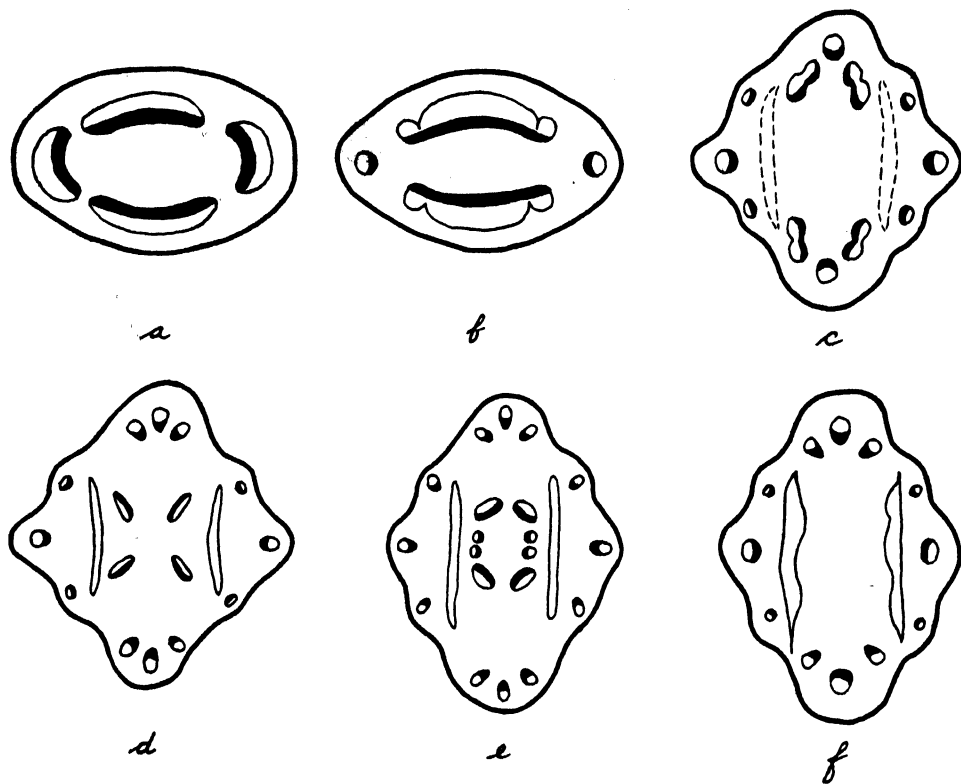
Miss Saunders' claim is that the four bundles remaining in the center of the receptacle after the departure of the uppermost stamen traces are the true carpal traces. This interpretation is incorrect as can be seen especially by the study of extra-carpel flowers, and (lest such be considered merely monstrous forms) of normal flowers in those species, *Conringia orientalis*, *Cheiranthus Allionii*, *Raphanus Raphanistrum*, and doubtless others, in which the stele of the receptacle continues *beyond the level of departure* of the traces to the existing carpels. That is, there are found in these plants, inside of the ring of "carpel traces," a ring of bundles, two or four, which continue upward for a short distance before dying out. These are vestigial strands, and form the remains of the upper part of the stele leading to one or more upper whorls of carpels, which are now lost.

The value of these strands is not merely as evidence of the former existence in the family of extra upper carpal whorls, but in demonstrating beyond question that Miss Saunders' interpretation of the four strands just below as carpal traces is incorrect and morphologically impossible.

These extra bundles are derived from the bundle which gives rise to the ventral traces of the fertile carpal (text fig. 8). That is, there remain, after the dorsal traces have been given off to the fertile carpels, two large stelar bundles. Each of these two bundles gives off from its edges the ventral traces of the fertile carpels; *the central portion of the bundle remains and passes upward, as a stelar bundle in the receptacle, swinging laterally*. The two large bundles from which these last bundles were derived cannot possibly be carpal traces as Miss Saunders claims for they could not then give rise to stelar bundles. It is possible, of course to dismiss them from consideration, as Miss Saunders has done with other disturbing bundles, by the teleological absurdity that they "are discards into the pith."

The same condition obtains in extra-carpel types, in some, at least, of those cases mentioned by Miss Saunders (4, p. 128) in "genera, which, now and again, produce 4-valved ovaries,"—where there are, in her words "4 residual portions of vascular tissue present above the level of departure of what she terms the four carpellary traces. These "residual portions" "also become cords." There is here much confusion of statement: the words "residual portions" surely imply that they are a part of the floral stele remaining after the departure of the bundles below. They "*become cords*," by which is evidently intended they "become traces of 4 more

carpels." (The fact that they "become cords" further implies that they were at first stelar.) Miss Saunders neglects to state *how they arise*,—the critical point, as she herself says. They actually arise at a forking (like the last forking in *Conringia*) when the ventral traces of the fertile carpels depart, either both at once from each side of the main bundle, or one somewhat before the other, leaving the "residual portion." The demonstration of how these arise could not be shown because her theory would



TEXT FIG. 8. Diagrams showing the origin of the carpel traces in *Conringia orientalis*, and showing proof that the four bundles at the base of the ovary (a) are not carpel traces, but true stelar bundles: a-c, as in typical crucifers (fig. 7), except that four stelar bundles remain after the second ventrals are formed (c); d, the stelar bundles form a cylinder and divide somewhat further (e); f, these vestigial stelar bundles have disappeared.

then fall, whether the "residual portions" are a part of the stele or are carpel traces. If these residual portions are stelar bundles then the ventral traces of the upper whorl of carpels are derived from them (this is indeed the true condition), and her so-called "lateral" bundles are not branches of the midrib bundle, but arise independently from the stele, as the authors claim. If the "residual portions" are true traces, they arise from what are, in her interpretation, lateral branches of a carpel; that is, carpel traces are derived from carpel traces, or leaves from leaves. The morphological impossibility of either interpretation is apparent.

In regard to the arrangement of the four carpels claimed by both Miss Saunders and the authors to be present in the Cruciferae, there is marked difference of opinion. The authors claim that there are two whorls of two, one distinctly above the other. Miss Saunders claims that the four are in one whorl. In regard to the authors' position in this feature, Miss Saunders says: "This statement (that there are two whorls of two carpels each) is at variance with the facts. The outward appearance of the gynoeceum might suggest that the valve carpels stand higher than the median pair, but could not possibly be interpreted in the opposite sense." The present writers, in spite of the proclaimed "impossibility," do so interpret the arrangement of the carpels, and insist that neither internal nor external structure is at variance with the facts, which show that the valve carpels are inserted below the fertile carpels. Not only is this externally evident, but the traces to the valve carpels leave the receptacular stele well before those to the fertile carpels (as has been stated before, p. 650) when judged by the test which is used both by Miss Saunders and the authors—"the level of emergence (from the floral stele) of the vascular cords." The difficulty rests in the interpretation of the bundles at the level in question; *i.e.* it is a matter of "When is a trace not a trace and a stelar bundle?" The authors cannot agree with Miss Saunders in this matter; they have shown wherein her interpretation is incorrect.

In a number of instances minor points are discussed by Miss Saunders in ways that seem to be quibbles over wording. The following will serve as examples of this criticism.

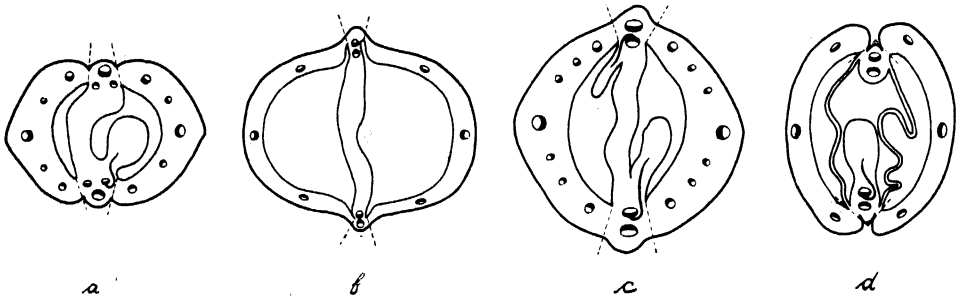
The authors, discussing the relation of the lower pair of carpels to the upper, have emphasized "the very unusual relationship of the lower carpels to this upper pair—a nearly complete enclosure." This statement is quoted by Miss Saunders and is followed by these words:

. . . and again on p. 261 they describe the upper set of carpels as having become enclosed by the lower set. But if 4 units together make up the circumference of a structure is it quite accurate to describe one pair of these units, even though they be smaller than the other pair, as being 'enclosed' by the latter? Presumably no two sectors of one circle can be considered as enclosing the other two. At what point or degree of inequality between the portions is "enclosure" deemed to occur? This unusual form of description (unless I have misunderstood the authors) seems to me to lead to fallacious arguments . . .

The authors, after definitely at first using the description quoted by Miss Saunders "a nearly complete enclosure," and giving figures of several genera from which it should be clear what is meant, have used the words "enclosure" and "enclosed" and "embraced" in reference to the relationship mentioned. It was not thought that after the relation had been described and figured as "a nearly complete enclosure," it would be necessary to modify the words "enclose" and "embrace" every time they were used by the phrase "nearly complete," and the authors doubt if the words have been misunderstood by readers. They submit that this enclosure of the two

upper carpels by the two lower is a striking feature of the Cruciferae and closely allied families (fig. 9), especially when the septum is considered, as it is by both Miss Saunders and the authors, as a part of the upper carpels; and they claim that the words "enclose" and "embrace" are proper and suitable words to describe the general condition, when the enclosure has first been described as "nearly complete."

Another quibble is as to whether the funicle is a part of the ovule. The



TEXT FIG. 9. Diagrams of typical ovary structure in crucifers and closely allied families to show the two pairs of carpels, and the fact that one pair nearly encloses the other: (a) *Barbarea vulgaris*; (b) *Camelina microcarpa*; (c) *Cheiranthus Cheiri*; (d) *Corydalis aurea*.

authors are said to have used "funicle" as though it "were not an integral part of the ovule"; and such usage is protested as also "likely to lead to fallacious arguments." An example cited is the statement that stomates occur upon the funicle but were not found upon the ovule. The meaning of the statement is obvious. Whatever the relation of the ovules to the carpels, both Miss Saunders and the writers discuss the funicles as extending through the septum, which is regarded by both as carpellary. In the opinion of the authors the septum represents the ventral portions of the upper carpels, from which the loculus has disappeared, and the ventral bundles have 'retreated' dorsally, during the reduction of the carpels. Miss Saunders, in calling attention to the fact that the authors have spoken of the funicle as though not a part of the ovule, calls attention also to the fact that she has freely discussed as the funicle that stalk-like base of the ovular body which extends through the tissues of the carpel to the "frame of the replum." She thus puts herself in the position of asserting indirectly the very thing the authors claim and she wishes to deny—that the ovule lies in part within the tissues of the carpel, and is hence "endogenous." The authors have emphasized this presence of the funicles within the replum as evidence of the extrusion of the ovules. They maintain that the ensheathment, often very loosely, of these funicles by the outer layers of the replum is important. Is not the very fact that there are funicles—distinct as such—*within the tissues* of the carpel body remarkable evidence that something at least is peculiar here in the attachment of the ovules? Miss Saunders apparently concedes the presence of funicles within the body

of the carpel, for she discusses them as such (4, pp. 124, 125), and the partition is considered by her a part of the fertile carpels. In no other families do the funicles of the ovules extend deep into the carpel tissues.

Finally, the very brief summary of Miss Saunders' paper (which does not summarize the content of the paper) grossly misrepresents the authors' position. It follows:

The median carpels of the Cruciferae are held by Eames and Wilson to be of a quasi-solid nature. In proof thereof they state that the ovules arise from these carpels endogenously, but neither the *a priori* argument which they develop nor the evidence which they allege in support of their views, is found, when further examined, to support this conclusion.

The introductory sentence is correct. In the following sentence it is said: "In proof thereof they state that the ovules arise endogenously—etc." This is definitely not the proof presented, as an examination of the authors' paper will show. The proof lies in the origin and course of the vascular bundles; as their summary (1, p. 268) states, "The evidence for this (the presence of two sterile and two fertile carpels) rests chiefly upon the vascular structure of the base of the ovary. The solid carpel, regarded by many as the placental region, is found to have the same vascular supply as other carpels. . . ."

The statement that the method of origin of the ovules is the proof presented in the authors' paper is clearly an erroneous statement of fact.

SUMMARY

1. This paper reaffirms the position of the authors in their previous paper dealing with the nature of the crucifer ovary. Additional evidence for their theory is presented, and the arguments of Miss E. R. Saunders, who has recently adversely criticized their position, are refuted.

2. From the evidence at hand it appears that there are four carpels in the Cruciferae instead of two, the generally accepted number. Two of these carpels are valve-like and sterile and are placed below two other carpels, nearly enclosing them. This second set of carpels, the fertile or "solid" carpels, is reduced; the loculus has disappeared, and the ovules borne by these carpels have been forced out of the loculus and lie in the loculus of the valve, or sterile, carpels. This has taken place phylogenetically, not ontogenetically. The writers believe that all four carpels are morphologically and anatomically the same; the "solid" carpel is a modification of a carpel of the same type as the sterile carpel.

3. In support of the above, the writers have again called attention to the nature of the vascular supply to both sterile and fertile carpels. This supply is normal for angiosperm carpels in general; it consists of a dorsal or midrib bundle and two marginal or ventral bundles to each carpel. The vascular supply to the ovules is derived from inversely oriented marginal or ventral bundles, a fact of great significance which Miss Saunders had

overlooked, and one to which she now refuses to accord importance. The position of the writers in respect to their belief that the ovules have pushed through the dissepiment and lie in the loculus of carpels to which they do not belong, is supported chiefly by the position of the marginal bundles and by the sheathing of the funiculus within carpellary tissue. They conceive no other interpretation of these facts to be supported by the facts of structure.

4. A number of Miss Saunders' statements are refuted, among which is the one that the ventral bundles are lateral branches of the dorsal bundle, rather than branches of truly stelar bundles; also that the sterile carpels are not placed below the fertile carpels, which the anatomical supply to these organs clearly shows to be the case.

5. On the evidence presented, either as applied to the Cruciferae or to other angiosperm families, the writers wish to place themselves on record as being opposed to the theory of carpel polymorphism. They believe Miss Saunders' position to be founded primarily upon a misapprehension of the true nature of the vascular supply to the angiosperm carpel.

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TRACING THE TRANSPIRATION STREAM WITH DYES

R. B. HARVEY

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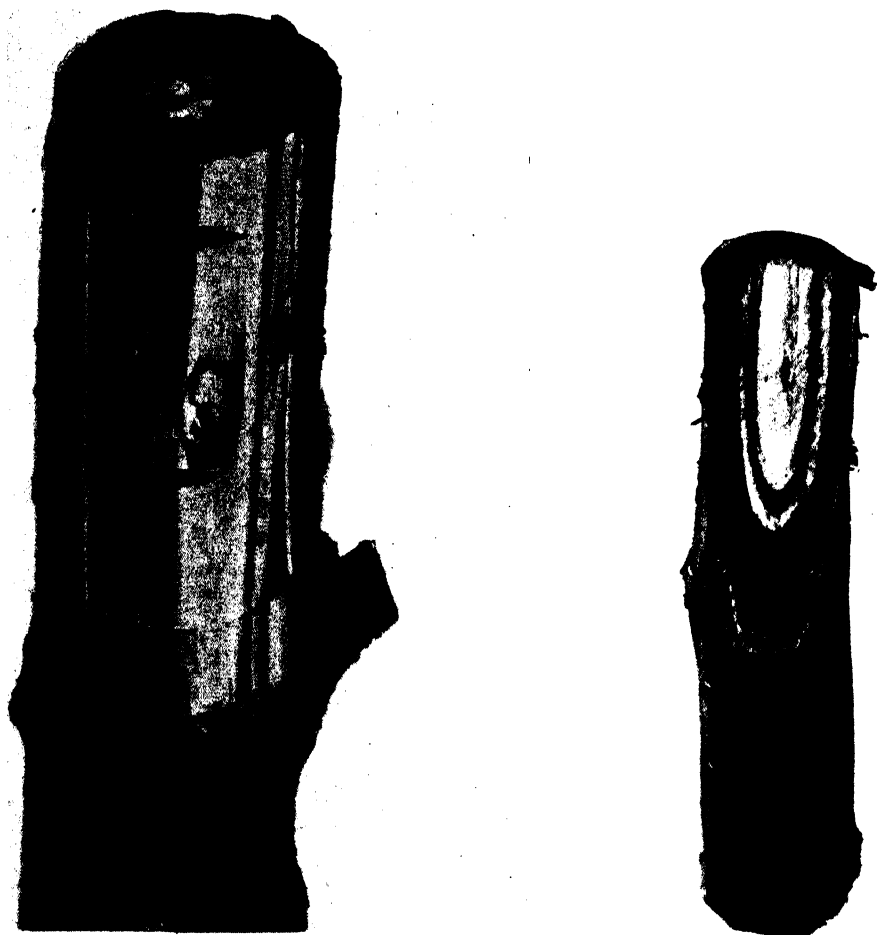
In teaching elementary plant physiology a common experiment for demonstrating the path of the transpiration stream is to immerse the ends of cut stems in dye solutions. Coulter (4) recommended the use of eosin solution. Arndt (1) also used eosin solution. When sections are made of the immersed stems, the place of conduction of the dye can be easily observed. But eosin and other such fluorescent dyes are relatively toxic substances, and frequently the stems must remain a long time in the solution to get good penetration. MacDougal, Overton, and Smith (5) used Acid Fuchsin and Orange G, but the author finds these substances more toxic to delicate tissues than Light Green S.F. and certain other dyes. Bergen and Davis (2) and Bodenbergl (3) use lithium salts, testing for the presence of lithium in the stem by spectroscopic means. To obviate the use of diffusible and toxic substances, Peirce (6) used suspensions of finely ground starch and obtained penetration of the starch through six inches of leaf stalk in half an hour. But this method requires that the starch shall be demonstrated through its reaction with IKI solution. All starch in the tissue will give the blue starch iodine reaction, and the localization is not as definite as desirable.

The author has found that Light Green S.F. gives a very fast penetration and is relatively non-toxic to plants. Using a solution of 1 gm. per liter of Light Green S.F., a green color can be observed in the petals of white sweet peas at a distance of one foot above the dye solution in from three to five minutes. This dye has been used for class demonstrations at the University of Minnesota for three years, with good success. Experiments may be completed in a single laboratory period or during lecture demonstrations. Light Green S.F. is not particularly toxic to plant tissues. Carnations, sweet peas, and calla lilies dyed with it to a deep green color will stay fresh for several days.

A few days before the opening of plum buds at University Farm in May, 1929, a root one-half inch in diameter was excavated, cut, and the end which was attached to the tree put into a jar of the dye containing 1 gm. per liter. The wood was dyed a deep bluish-green and when the buds opened, the flowers were brilliant bluish-green. The flowers lasted for a normal time. Fruits were set and developed normally. When the leaf buds expanded, the leaves were dyed, and the bluish-green could be seen easily before the chlorophyll was fully developed. Later the bluish-green color of the dyed leaves could be noticed in sunlight. The dyed leaves grew to normal size

and on September 1 gave no evidence of injurious effect of the Light Green S.F.

The dye was conducted in the plum tree nine feet in forty-eight hours. The conduction for a few feet above the ground was localized in the trunk on the side immediately above the root which had been immersed in the dye; but, owing to the twisting of the trunk, the limb which bore the most dye was on the opposite side of the crown from the immersed root. The



TEXT FIG. 1 (Left). Box elder stem showing the distribution of Light Green S.F. Green is shown as black in the figure. TEXT FIG. 2 (Right). Plum branch showing the places of conduction in relation to the annual rings.

path of the dye through the xylem could be nicely traced in cut sections. In white pine, black spruce, cotton-wood, and linden a similar localization of the dye in the xylem connecting with the immersed root was observed. There is evidently much more rapid conduction upward through the xylem

than tangentially around the trunk or between the annual rings, so that the position of the xylem which connects with the particular root immersed is clearly marked with a streak of dye.

When a branch was cut off and the end attached to the tree was immersed in dye solution, the wood, flowers, and leaves on this branch were deeply colored with dye. In this case the dye was conducted back to the trunk and again up to branches to a distance of several feet. Evidently conduction was not limited to the outward direction, and the dye was pulled in through the cut end of the limb.

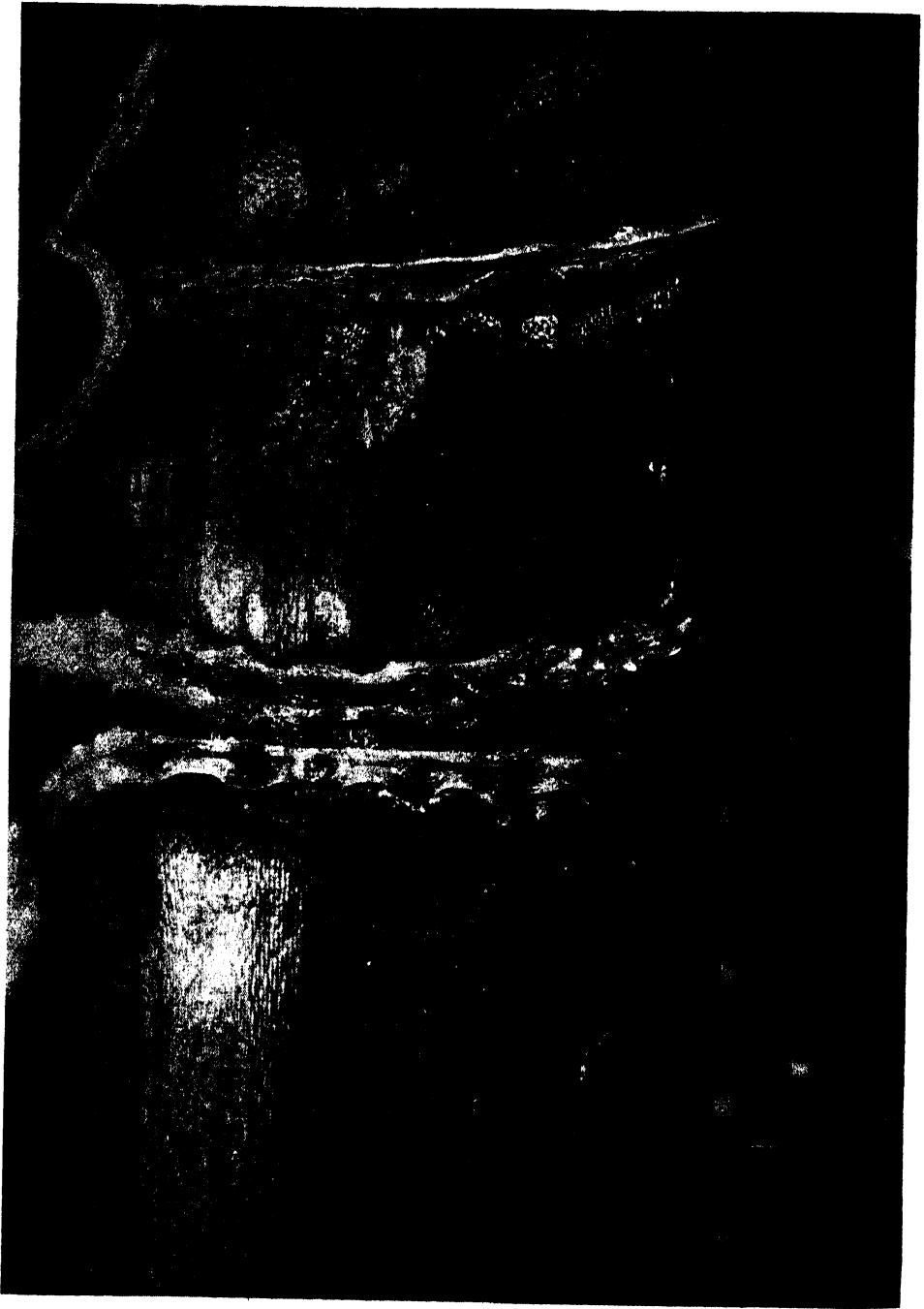
In all of the cases observed where branches of pine, cotton-wood, apple, plum, tomato, and geranium were cut off and set into the dye solutions, conduction took place through the wood only. The bark showed no presence of dye. The isolation of bark and wood can be seen in text figure 1, in which the wood of box elder is stained deep green and the green solution can be centrifuged or squeezed from it, while the bark has a layer of only faintly dyed cells at the cambium. The place for the transfer of the dye from wood to bark can be seen on the colored portion of the wood, in which it corresponds with the medullary rays. In this photograph there is shown also the effect of two slightly bruised spots upon the conduction in the outer wood. There is a narrow strip of functioning xylem between the two bruises, and the effect of the bruise is limited to a layer 1/16 inch deep. The lack of conduction in the pith should be noted.

In a section of plum stem, text figure 2, there is considerable difference in the amount of dye solution in the different rings of the wood. The darkest green area corresponded to the wood laid down during the early summer of 1927, and to less degree in 1928 and 1926. In the groups of very large tracheae laid down in the spring of 1926, 1927, and 1928 there is much dye; then in the later spring wood the color is less. In the early summer wood the conduction is again increased and is followed by an area of decreased conduction. The wood laid down before 1926 scarcely conducts at all.

In white pine branches the conduction is uniform throughout the wood and shows little variation with the annual rings. In corn stalk the conduction of the dye solution is sharply limited to the xylem elements. In squash stem there is conduction of dye in the xylem and also in the collenchyma cells at the angles of the stems.

The Light Green S.F. penetrates along the xylem very rapidly. It diffuses from the tracheae and tracheids only very slowly, if at all. Preparations of petals of white flowers show distinctly the distribution of the tracheae and ramifications of the conducting vessels. In fruits the use of this dye is of value for demonstrating the path of transport in the transpiration stream through carpels and into the seed. The cutting off of fruits and leaves by abscission layers can be demonstrated by failure of the dye to penetrate beyond the abscission layer.

The relation between the xylem systems of host and of parasite can



TEXT FIG. 3. The conduction of Ponceau 3R from buckwheat stem into dodder. Note the dye (in black) in the xylem of the parasite, especially at the haustorial connections.

be demonstrated easily by use of non-toxic dyes. Buckwheat and dodder seeds are sown together in flats. When the buckwheat plants are a foot high, the dodder should be firmly attached by several wrappings around the stem of the host. When the parasite has become established, its connection to the soil or other plants is cut off and allowed to heal over. Then the buckwheat stem is cut off and put into the dye solution. The dye rapidly penetrates to all parts of the xylem of the host. The coloration of the xylem of the dodder indicates that there is a direct connection between the parasite and the host. The dye solution does not usually pass from the xylem elements of the buckwheat into other cells, yet it passes freely into the xylem of the parasite. The passage through the haustoria is easily seen in section as shown in text figure 3.

Dyes of other colors may be used also, especially the following:

| | |
|----------------|--------------|
| Brilliant Blue | Blue |
| Tartrazine | Lemon yellow |
| Ponceau 3R | Purplish red |
| Amaranth | Purplish red |

SUMMARY

Light Green S.F., Tartrazine, Brilliant Blue, Amaranth, and Ponceau 3R were found to be useful for demonstrating the path of the transpiration stream. When the xylem is opened by cutting off root, stem, or branch, these dyes quickly penetrate the wood. Localization in the xylem elements is good since the dyes do not diffuse out very much from the tracheae and tracheids.

These dyes are non-toxic. Cut sweet peas and carnations live for a normal time although the petals are dyed deeply. The colors can be mixed, giving practically the whole spectral range. Penetration is fast enough so that a lecture-table demonstration can be made for classes.

Localization of conduction in the wood is easily demonstrated, the depth of color indicating the volume of flow in the annual rings. The conduction from host to parasite also can be shown.

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THE TAXONOMIC AND CLIMATIC DISTRIBUTION OF OIL AND STARCH IN SEEDS IN RELATION TO THE PHYSICAL AND CHEMICAL PROPERTIES OF BOTH SUBSTANCES

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The object of this paper is to show the climatic and taxonomic distribution of oil and starch in seeds and to point out a possible relationship between the climatic distribution of both substances and their physical and chemical properties.

Nägeli, in 1858, showed the distribution of oil and starch in the seeds of many species and families of plants. His data forms the basis for this paper. Before it could be used, however, it was necessary to revise the species and families listed by him in accordance with Engler and Prantl and the Kew Index. The climatic distribution of the families used is that given by Engler and Prantl. Nägeli did not correlate oil and starch distribution with climate.

PHYSICAL AND CHEMICAL PROPERTIES OF OIL AND STARCH IN RELATION TO THEIR DISTRIBUTION

An explanation for the predominance of oil in some seeds and the predominance of starch in others may perhaps be found in the chemical and physical relationships between these two substances, the climatic environment during their formation, and the structure of the seeds in which they occur.

As oil conducts heat less readily than starch, it may therefore be of service in the seed as a protection against cold in temperate climates or against excessive heat in the tropics.

Oil is far lighter than starch in weight (specific gravity of starch 1.56, oil $0.98 \pm$), and should be of benefit in the dispersal of seed by streams or wind.

Assuming the nutritive value of starch and oil to be equal, weight for weight, oil would be more compact owing to the difference between their specific gravities. In accordance with this difference in density oil would only occupy 70 percent of the space occupied by an equal weight of starch, and all other factors being equal, small seeds might be expected to contain oil and large seeds starch.

Oil has a greater fuel value (1 g. carbohydrate gives 4.1 calories, 1 g. oil gives 9.3 calories) and as oil before utilization apparently is changed into carbohydrates (an exothermic reaction), it follows that 1 g. of oil

corresponds to 1.6 g. of carbohydrate if directly oxidized to glucose. Oil in consequence of its greater heating value should aid seeds in germinating in cool localities. In accordance with the heat conductivity and fuel value of oil it might be expected that seed embryos, leaf and flower buds, and bulbs might be richer in oil than in starch.

In accordance with the facts expressed above: tropical seeds and seeds of arctic plants would be likely to be more oily than starchy (difference in heat conductivity); seeds disseminated by water and wind more likely to be oily than starchy (difference in specific gravity); seeds disseminated by birds more likely to be oily than starchy (such seeds are of necessity small); seeds germinating in cool localities, e.g., early spring in temperate climates, would more likely be oily than starchy (difference in fuel value).

DISTRIBUTION OF OIL AND STARCH IN SEEDS

Taxonomic. Data of the starch and oil content of the seeds of 216 families of the 280 families (angiosperms and gymnosperms) listed by Engler and Prantl has been examined (table 1). Of the seeds of these 216 families 139, or 64.35 percent, contain oil and no starch, 52 or 24.07 percent, contain both oil and starch, and 15, or 6.94 percent, contain starch alone.

Where oil and starch are specified by Nägeli as contents of the embryo, 80, or 37.04 percent, contain oil and no starch, 21, or 9.72 percent, contain both oil and starch, and 10, or 4.63 percent, contain only starch.

Where the content of the albumen (endosperm and perisperm) in starch and oil is specified, of the 216 families, 54, or 25.00 percent, contain oil and no starch, 14, or 6.48 percent, contain both starch and oil, and 30, or 13.89 percent, contain starch with no oil.

Nägeli states that when starch occurs in seeds, other reserve food is almost entirely absent; if seeds which are rich in starch have albumen, then the embryo with few exceptions contains oil and no starch; but if albumen is absent from the seeds, the starch is found in the cotyledons, while oil alone is usually present in the cells of the caulicle and plumules. Whenever the various genera of an order differ in this respect, this difference will occur usually according to the sizes of their respective seeds, starch being found in the large seeds if not in the small seeds.

Climatic. Of the 216 families examined 82 are tropical, 29 are tropical and subtropical, 24 are subtropical, 4 are subtropical and temperate, 41 are temperate, and 36 are widely distributed (table 1).

The climatic distribution of starch and oil in these seeds is as follows: Of the 82 tropical seeds 22, or 26.83 percent, contain both starch and oil, 8, or 9.76 percent, contain starch with no oil, and 50, or 60.90 percent, contain oil but no starch. Of the 29 seeds that come from families which occur mostly in tropical and subtropical zones, 8, or 27.59 percent, have both starch and oil as components, 3, or 10.34 percent, have starch but no oil, and 18, or 62.07 percent, have oil but no starch. Of the 24 plant

TABLE 1. *Climatic and Taxonomic Distribution of Oil and Starch in Seeds*

| Family | Embryo | | Albumen | | General Contents | Family | Embryo | | Albumen | | General Contents |
|--------------------|--------|-----|---------|-----|------------------|---------------------|--------|-----|---------|-----|------------------|
| | Starch | Oil | Starch | Oil | | | Starch | Oil | Starch | Oil | |
| Cycadaceae †† | + | o | + | o | Oily | Aristolochiaceae †† | o | — | o | — | Oily |
| Taxaceae *† | o | — | o | — | | Rafflesiaceae † | o | — | o | — | Oily |
| Pinaceae * | o | — | o | — | | Polygonaceae * | o | + | + | — | Oily |
| Gnetaceae * | o | + | + | + | | Chenopodiaceae § | o | + | ± | — | |
| Typhaceae †* | o | + | + | + | | Amarantaceae † | o | + | + | — | |
| Pandanaceae † | o | + | o | + | | Ceratophyllaceae § | + | + | — | — | |
| Najadaceae * | + | + | — | — | | Nyctaginaceae § | o | + | + | — | |
| Aponogetonaceae † | o | o | + | o | | Cynocrambaceae † | o | — | o | — | Oily |
| Alismaceae § | + | + | — | — | | Phytolaccaceae †† | o | + | ± | — | Oily |
| Butomaceae †* | + | o | — | — | | Aizoaceae †† | o | + | + | — | Oily |
| Hydrocharitaceae § | + | + | — | — | | Portulacaceae †† | o | + | + | — | |
| Gramineae § | o | + | + | ± | Oily | Caryophyllaceae * | o | + | + | — | |
| Cyperaceae § | o | + | + | + | | Nymphaeaceae † | o | + | + | — | |
| Palmae † | o | + | o | + | | Ranunculaceae * | o | + | o | + | |
| Araceae † | o | + | + | o | | Berberidaceae * | o | — | o | — | Oily |
| Lemnaceae § | o | — | o | — | | Menispermaceae †† | o | — | o | — | Oily |
| Flagellariaceae † | — | — | + | — | | Magnoliaceae †* | o | — | o | — | Oily |
| Restionaceae † | — | — | + | — | | Calycanthaceae * | — | — | — | — | Oily, some st. |
| Centrolepidaceae † | o | + | + | o | Oily | Anonaceae † | o | — | o | — | Oily |
| Xyridaceae † | — | — | + | — | | Myristicaceae † | o | + | + | + | Oily |
| Eriocaulaceae † | — | — | + | — | | Monimiaceae †† | o | + | o | + | |
| Bromeliaceae †† | — | — | + | — | | Lauraceae † | + | + | — | — | |
| Commelinaceae †† | — | — | + | — | | Hernandiaceae † | ± | + | o | + | |
| Pontederiaceae †† | o | + | + | — | | Papaveraceae * | o | — | o | — | Oily |
| Philydraceae † | — | — | + | — | | Cruciferae * | o | — | o | — | Oily |
| Juncaceae * | o | + | ± | + | | Capparidaceae †† | o | — | o | — | Oily |
| Liliaceae § | o | + | o | + | | Resedaceae † | o | — | o | — | Oily |
| Haemodoraceae † | o | — | + | — | | Moringaceae † | o | + | — | — | Oily |
| Amaryllidaceae †† | o | — | o | + | Oily | Sarracenaceae § | o | — | o | — | |
| Taccaceae † | o | — | o | — | Oily | Nepenthaceae † | o | + | + | + | |
| Dioscoreaceae †† | o | — | o | — | Oily | Droseraceae § | o | + | + | ± | |
| Iridaceae § | o | — | o | + | Oily | Podostemaceae † | o | + | o | + | |
| Musaceae † | — | — | + | — | | Crassulaceae * | o | — | o | — | Oily |
| Zingiberaceae † | o | + | + | — | | Saxifragaceae * | o | — | o | + | Oily |
| Cannaceae †† | o | + | + | — | | Pittosporaceae † | o | — | o | — | Oily |
| Burmanniaceae † | o | — | o | — | Oily | Cunoniaceae † | o | — | o | — | Oily |
| Orchidaceae † | o | — | o | — | Oily | Bruniaceae † | o | — | o | — | Oily |
| Casuarinaceae †† | o | — | o | — | Oily | Hamamelidaceae *† | o | + | o | + | Oily |
| Piperaceae † | — | — | + | — | Oily | Platanaceae * | o | — | o | — | |
| Chloranthaceae † | o | — | o | — | Oily | Rosaceae * | o | — | o | — | |
| Salicaceae * | o | — | o | — | Oily | Connaraceae † | o | — | o | — | |
| Myricaceae * | o | — | o | — | Oily | Leguminosae § | ± | + | + | + | |
| Juglandaceae * | o | — | o | — | Oily | Geraniaceae § | o | + | o | + | |
| Betulaceae * | o | — | o | — | Oily | Oxalidaceae †† | o | — | o | — | |
| Ulmaceae § | o | — | o | — | Oily | Tropaeolaceae † | o | — | o | — | |
| Fagaceae * | o | — | o | — | Oily | Linaceae * | o | + | o | + | |
| Moraceae † | o | — | o | — | Oily | Humiriaceae † | o | + | o | + | |
| Urticaceae † | o | — | o | — | Oily | Zygophyllaceae †† | o | + | o | + | |
| Proteaceae †† | o | — | o | — | Oily | Erythroxylaceae † | ± | ± | ± | ± | Oily |
| Loranthaceae † | + | — | + | — | Oily | Rutaceae † | ± | + | o | + | |
| Santalaceae †* | o | + | o | + | | Simarubaceae † | o | + | o | + | |
| Grubbiaceae † | o | — | o | — | | | | | | | |
| Opiliaceae † | o | + | o | + | | | | | | | |

‡ Mostly tropical.

* Mostly temperate.

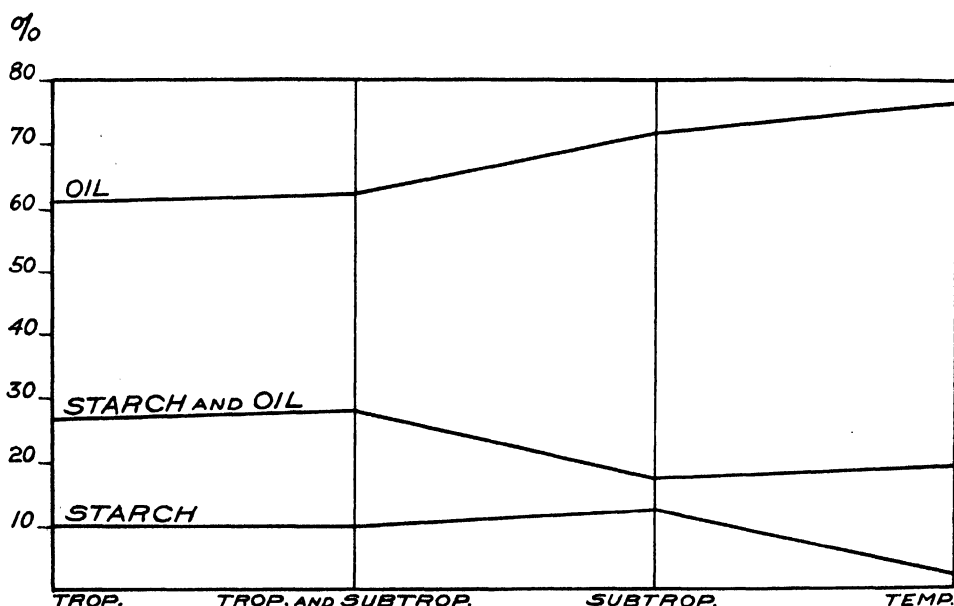
† Mostly subtropical.

§ Widely distributed.

TABLE 1.—Continued

| Family | Embryo | | Albumen | | General Contents | Family | Embryo | | Albumen | | General Contents |
|--------------------|--------|-----|---------|-----|------------------|--------------------|--------|-----|---------|-----|------------------|
| | Starch | Oil | Starch | Oil | | | Starch | Oil | Starch | Oil | |
| Burseraceae † | o | — | o | — | Oily | Pimicaceae † | o | + | — | — | |
| Meliaceae †† | ± | + | o | + | | Rhizophoraceae † | o | — | — | — | |
| Malpighiaceae † | o | — | o | — | Oily | Combretaceae † | o | — | o | — | Oily |
| Vochysiaceae † | o | — | o | — | Oily | Myrtaceae †† | ± | o | o | o | |
| Tremandraceae † | o | + | o | + | | Melastomataceae † | ± | ± | — | — | |
| Polygalaceae § | o | — | o | — | Oily | Onagraceae * | o | — | o | — | Oily |
| Dichapetalaceae † | + | ± | + | ± | | Hydrocaryaceae † | + | — | — | — | |
| Euphorbiaceae † | o | + | o | + | | Halorrhagidaceae § | o | + | o | + | |
| Callitrichaceae § | o | — | o | — | Oily | Cynomoriaceae † | o | — | o | — | Oily |
| Coriariaceae * | o | — | o | — | Oily | Araliaceae †† | o | — | o | — | Oily |
| Empetraceae * | o | — | o | — | Oily | Umbelliferae * | o | + | o | + | |
| Limnanthaceae * | o | — | o | — | Oily | Cornaceae * | o | + | ± | + | |
| Anacardiaceae † | ± | + | o | — | Oily | Pirolaceae * | o | — | o | — | Oily |
| Aquifoliaceae § | o | + | o | + | | Ericaceae * | o | — | o | — | Oily |
| Celastraceae § | o | + | o | + | | Epacridaceae † | o | — | o | — | Oily |
| Hippocrateaceae † | o | + | — | — | | Diapensiaceae * | o | — | o | — | |
| Stackhousiaceae † | o | + | o | + | | Myrsinaceae † | + | — | — | — | Oily |
| Staphyleaceae * | o | + | o | + | | | | | | | ± |
| Aceraceae * | ± | + | ± | + | | Primulaceae * | o | — | o | — | Oily |
| Hippocastanaceae * | + | — | — | — | | Plumbaginaceae § | o | + | + | — | |
| Sapindaceae † | ± | ± | o | + | | Sapotaceae † | ± | + | o | + | |
| Melanthaceae † | o | — | o | — | Oily | Ebenaceae †† | o | — | o | — | Oily |
| Balsaminaceae † | o | — | o | — | Oily | Styracaceae †† | o | — | o | — | Oily |
| Rhamnaceae § | o | + | o | + | | Oleaceae †* | o | + | o | + | |
| Vitaceae † | o | — | o | — | Oily | Salvadoraceae † | o | — | o | — | Oily |
| Elacocarpaceae † | o | — | o | — | Oily | Loganiaceae † | o | + | o | + | |
| Tiliaceae † | o | + | o | + | | Gentianaceae § | o | — | o | — | Oily |
| Malvaceae † | o | — | o | — | Oily | Apocynaceae † | o | — | o | — | Oily |
| Bombacaceae † | ± | + | o | + | | Asclepiadaceae † | o | + | o | + | |
| Sterculiaceae † | ± | + | ± | + | | Convolvulaceae † | ± | + | o | + | |
| Dilleniaceae † | o | — | o | + | | Polemoniaceae * | o | + | o | + | |
| Ochnaceae † | ± | — | o | — | Oily | Hydrophyllaceae * | o | + | o | + | |
| Caryocaraceae † | o | — | o | — | Oily | Borraginaceae †* | o | — | o | — | Oily |
| Marcgraviaceae † | o | — | o | — | | Verbenaceae †† | ± | + | o | + | |
| Guttiferae † | ± | + | o | — | | Labiatae †* | o | — | o | — | Oily |
| Dipterocarpaceae † | + | — | — | — | | Solanaceae † | o | + | o | + | |
| Elatinaceae § | o | — | o | — | Oily | Scrophulariaceae § | o | + | o | + | |
| Frankiniaceae † | — | + | + | — | | Bignoniaceae † | o | — | o | — | Oily |
| Tamaricaceae † | o | + | ± | — | | Martyniaceae †† | o | — | o | — | Oily |
| Cistaceae † | o | + | + | — | | Orobanchaceae * | o | — | o | — | Oily |
| Cochlospermaceae † | o | + | o | + | | Gesneraceae †† | o | — | o | — | Oily |
| Canellaceae † | o | + | o | + | | Lentibulariaceae † | o | — | o | — | Oily |
| Violaceae § | o | — | o | — | Oily | Globulariaceae † | o | — | o | — | Oily |
| Flacourtiaceae † | o | + | o | + | | Acanthaceae † | o | — | o | — | Oily |
| Turneraceae † | o | — | o | — | Oily | Myoporaceae † | o | — | o | — | Oily |
| Malesherbiaceae † | o | + | o | + | | Plantaginaceae § | o | + | o | + | |
| Passifloraceae † | o | — | o | — | Oily | Rubiaceae † | o | + | o | + | |
| Caricaceae †† | o | + | o | + | | Caprifoliaceae †† | o | + | o | + | |
| Loasaceae †† | o | + | o | + | | Valerianaceae * | o | — | o | — | Oily |
| Datisceae * | o | — | o | — | Oily | Dipsacaceae † | o | + | o | + | |
| Begoniaceae † | o | — | o | — | Oily | Cucurbitaceae † | o | — | o | — | Oily |
| Cactaceae †† | o | + | o | — | | Campanulaceae * | o | + | o | + | |
| Penaeaceae † | o | — | o | — | Oily | Goodeniaceae † | o | + | o | + | |
| Oliniaceae †† | o | — | o | — | Oily | Stylidiaceae † | o | — | o | — | Oily |
| Thymelaeaceae § | o | — | o | — | Oily | Calyceaceae † | o | + | o | + | |
| Elaeagnaceae * | o | — | o | — | Oily | Compositae § | o | — | o | — | Oily |
| Lythraceae † | o | — | o | — | Oily | | | | | | |

families whose habitats are mainly subtropical, 4, or 16.66 percent, have both oil and starch, 3, or 12.50 percent, have starch but no oil, and 17, or 70.84 percent, have oil but no starch. The 41 plant families whose habitats are for the most part temperate have seeds with compositions as follows: 8, or 19.51 percent, contain both starch and oil, 1, or 2.44 percent, have starch but no oil, and 31, or 74.61 percent, have oil but no starch. Of the 36 families whose habitats are widely distributed climatically 11, or 30.55 percent, have both starch and oil in their seeds, 1, or 2.77 percent, has starch but no oil, and 23, or 63.89 percent, have oil but no starch. These percentages demonstrate a definitely increasing proportion of oil from a minimum in tropical seeds to a maximum in seeds of temperate



TEXT FIG. 1. Climatic distribution of starch and oil in seeds.

plants. Conversely the starch content of the seeds is less in seeds of temperate climate than in seeds of the tropics. These trends are illustrated in text figure 1.

It is of interest to compare the analyses of the percentages of starch and oil in the seed embryos. Of the 42 embryos of tropical seeds 13, or 30.95 percent, contain starch and oil, 5, or 11.90 percent, contain starch with no oil, and 24, or 57.14 percent, have oil with no starch. The 14 embryos of tropical and subtropical seeds have contents as follows: 2, or 14.28 percent, have both starch and oil, an equal number have starch but no oil, and 10, or 71.43 percent, have oil but no starch. The 11 subtropical embryos do not have starch and oil occurring as joint constituents, but 1, or 9.09 percent, has starch with no oil and 10, or 90.91 percent, have oil with no starch. Temperate embryos number 15, 2 or 13.33 percent of

which contain both starch and oil, while 1, or 6.66 percent, has starch with no oil and 12, or 80.00 percent, have oil but no starch. The 27 embryos whose families have habitats of wide climatic distribution have 4, or 3.70 percent, containing both starch and oil, 1, or 14.81 percent, with a content of starch without oil, and 22, or 81.48 percent, that contain oil but no starch. These data parallel those of the analyses of entire seeds in the decrease in the starch content from tropical to temperate seeds and an increase in oil content from tropical to temperate seeds.

An examination of the seed albumen contents gives the following data: Of the 38 tropical albumens 5, or 13.15 percent, contain both starch and oil, 11, or 28.95 percent, contain starch without oil, and 22, or 57.90 percent have oil but no starch. Of the 15 tropical and subtropical family embryos, none have both starch and oil, 8, or 53.33 percent, have starch but no oil, and 7, or 46.66 percent, have oil but no starch. Of the 11 albumens of subtropical families, none has both starch and oil as contents, 6, or 54.54 percent have starch but no oil, and 5, or 45.45 percent, have oil but no starch. Of the families of temperate habitat 14 albumens are divided as follows: 4, or 28.57 percent, have both starch and oil, 2, or 14.28 percent, have starch but no oil, and 8, or 57.14 percent, have oil but no starch. The 18 seed albumens of families with widely climatically distributed habitats have 5, or 27.77 percent, with both starch and oil, 3, or 16.66 percent, with starch only, and 10, or 55.55 percent, with oil only. These results indicate a decrease from tropical to temperate habitat in the percentage of seed albumens which contain starch without oil. This indication is the reverse of that found in the examination of embryos and entire seeds. However, when both the percentages of starch and starch and oil containing groups are considered, namely, 13.15 percent and 28.95 percent for tropical albumens and 28.57 percent and 14.28 percent for temperate albumens, the results are more harmonious. It is evident, too, that the smaller number of albumens analyzed, 78, is probably less reliable than the larger number of entire seeds, 176. This may account for the lesser regularity in the analyses of the albumens than occurs in the trends of the analyses of the entire seeds.

CONCLUSIONS

Observation of the percentages obtained above indicates that because a large percentage of oil occurs in the seeds of tropical and temperate plants it may function as a protection against rapid temperature changes. The increased frequency of oil in the seeds of temperate plants is in accordance with the increased fuel value of oil over starch.

SUMMARY

The families of plants whose seeds contain oil and starch as listed by Nägeli have been revised in accordance with data from Engler and Prantl. They number 216 families. The taxonomic and climatic distribution of starch and oil in these seeds has been determined.

Oil is more abundant than starch in seeds and in the seed embryos and albumens.

In the seeds of temperate plant families starch is present less frequently than in the seeds of tropical plant families.

The large proportion of oil in the seeds of tropical and temperate plants may indicate its function as a protection against rapid temperature changes in accordance with its heat conductivity which is lower than that of starch. The larger proportion of oil to starch in the seeds of temperate plants is in agreement with the greater fuel value of oil.

The greater frequency of oil than starch in seeds may aid in their dispersal by streams (lower specific gravity of oil) and their dispersal by birds (oily seeds are generally smaller than starchy).

FIELD MUSEUM OF NATURAL HISTORY,
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A PORTABLE PLANT DRIER FOR TROPICAL CLIMATES

L. H. MACDANIELS

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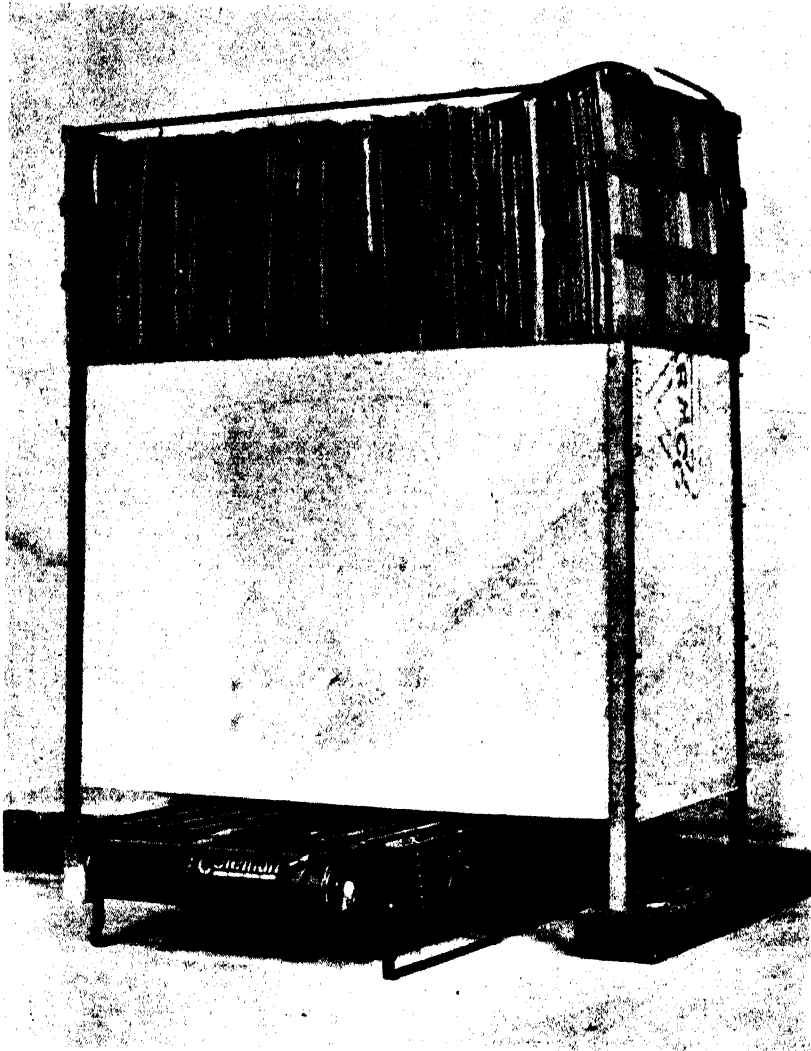
Anyone who has attempted to make herbarium specimens in the tropics where the humidity is high and rains frequent has encountered the difficulty of drying plants quickly and satisfactorily under such conditions. In some cases if one is near a town, it may be possible to place the plant presses on the top of the village bake-oven, over the boilers of a sugar mill, or in some other suitable place. Away from settlements, however, this is impossible and the botanist is obliged to resort to some makeshift contrivance, such as placing the presses on green poles over slow fires or using the heat from kerosene lamps.

After considerable unsatisfactory experience, the drying outfit illustrated herewith (text fig. 1) was devised. It consists of two pieces of heavy galvanized sheet-iron three feet by two, and two pieces one and one-half feet by two, with about one-half inch of their upper and lower edges turned over at right angles to increase their rigidity. These are fastened with small stove bolts to four angle-iron posts two and one-half feet long which also form the legs of the apparatus. This makes a rigid compartment or chimney two feet high, three feet long, and eighteen inches wide standing upon legs six inches high.

A satisfactory source of heat is a two-burner gasoline camp-stove of the type with a small pressure fuel-tank that is blown up with an attached pump. The press used is of the standard type with thick paper-felt blotters, corrugated pasteboard ventilators, and somewhat pliable press boards. In pressing plants, the specimen is placed in folded newsprint and placed between two of the blotters. A ventilator is placed between each pair of blotters, and the press built to any length up to three feet. Pressure is applied by means of the canvas trunk-straps. The press should be placed upon the top of the box-like chimney, so that no heat can escape except through the ventilators. If the press is too short to cover the whole top of the drier the openings can be closed with some of the blotters laid out flat over them.

For best results both burners should be going at full capacity at the start for about fifteen minutes or half an hour. During this time, the press can be turned over so as to get it heated throughout. When it is thoroughly hot a current of air will be set up through the ventilators of the press. The burners may then be turned down somewhat. After about an hour the straps will become loose because of the wilting of the plants, and should be tightened.

With this outfit, it is possible to dry most plants within twelve hours although some of the more resistant species may require twice that time. The collector can return to his base after several days collecting with field presses and vasculum full of plants and have them all dried within



from twenty-four to thirty-six hours. The apparatus is easily taken down by removing the stove bolts and so can readily be transported in small space wherever boats, railroads, or pack animals can travel. It is somewhat awkward and heavy to be carried far by man power.

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DIFFERENTIAL GROWTH OF PHYTOPHTHORAS UNDER THE ACTION OF MALACHITE GREEN ¹

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(Received for publication March 10, 1930)

INTRODUCTION

Differential growth is often employed to identify strains of certain difficult species of bacteria and fungi. While not always a sure criterion, such growth reactions may often possess a pertinent cumulative value, provided that the right sort of inhibitive substances be used. If the critical concentration of such substances happens to be rather rigid and narrow, permitting no large numbers of dilutions at which members of a given group of organisms may grow or fail to grow, less satisfactory results can be expected, whereas a relatively broader margin of growth tolerance is conducive to more clear-cut differentiations. Malachite green is a substance of the latter type, as it is able to induce differential growth in *Phytophthora* when its concentration is varied from one part of the dye in one million parts of nutrient solution to one part in sixteen million. Such a comparatively wide margin tends to create some fairly well-defined spheres where numerous species and strains may aggregate themselves into tentative groups.

The toxic effect of malachite green upon certain fungi was tested by Coons ² who found that this dye in dilutions of 1 part in 40,000 was able to prevent growth in a number of species. The writer ³, working with 220 different cultures of *Fusarium*, found that a number of them failed to grow in presence of 0.0002 percent malachite green, and only three organisms were able to make any growth when the concentration of this dye was increased to 0.01 percent.

It is the purpose of this paper to present the effects of malachite green upon the species of *Phytophthora*, tracing their fluctuating limits on the one hand and their possible interspecific relationships on the other.

A taxonomic work constructed upon the morphology or the physiology of a single organism may prove highly inadequate and misleading. With this idea in view, the writer endeavored to secure as many different strains of *Phytophthora* species as possible; but, with the exception of *P. cactorum* and

¹ Scientific paper No. 85 of the West Virginia Agricultural Experiment Station. The writer is indebted to Mr. Russell G. Brown for assistance in the laboratory.

² Coons, G. H. Some aspects of the *Fusarium* problem. Plant pathology and physiology in relation to man. Mayo Foundation Lectures, 1926-27.

³ Leonian, L. H. Studies on the variability and dissociations in the genus *Fusarium*. Phytopathol. 9: 753-868. Pls. 16-32. 1929.

P. omnivora groups, all of the remaining species were available only in limited numbers of strains.

Except in a few instances, the nomenclature has not been changed; many of the organisms listed here have no status as true species, but are retained under their original names in order to make comparison easier.

ORGANISMS STUDIED

- P. cactorum* (Lebert and Cohn) Schröt, strains 1, 2, 3, 4 and 5 were sent by Charles Drechsler who isolated them from *Lilium pyrenaicum*, *L. candidum*, *L. washingtonianum*, *L. speciosum*, and *L. regale*, respectively.
- P. cactorum* 6, 7 and 8 were sent by M. W. Gardner who isolated them from the apple fruit.
- P. cactorum* 9 is Beach's rhubarb strain, sent by C. M. Tucker.
- P. cactorum* 11, isolated by Beach from the apple fruit, was sent by Tucker.
- P. cactorum* 12 and 13, isolated from the apple fruit, was sent by Tucker.
- P. cactorum* 14, the Baarn strain.
- P. cactorum* 16, sent by E. W. Blackwell, Englefield Green, England.
- P. cactorum* 19, isolated from the apple fruit by the writer.
- P. cactorum* 20, sent by E. L. Felix.
- P. cactorum* 21, 23, 24 and 25 represent the respective strains of Peter, Rose, Meurs, and Sawada, and were obtained from Baarn.
- P. cambivora* (*Blepharospora cambivora* Petri), obtained from Baarn.
- P. capsici* Leon., isolated from pepper by the writer.
- P. citrophthora* (*Pythiacistis citrophthora* Smith), sent by H. S. Fawcett.
- P. citricolum* Saw., obtained from Baarn.
- P. cinnamomi* Rands 1, obtained from Rands.
- P. cinnamomi* 2, sent by Tucker who isolated it from *Persea gratissima* in Porto Rico.
- P. cinnamomi* 3, sent by R. P. White who isolated it from *Rhododendron* in New Jersey.
- P. colocasiae* Racib., secured from McRae, Pusa, India.
- P. cryptogea* Pethyb. and Laf., obtained from Baarn.
- P. erythroseptica* Pethyb., obtained from Baarn.
- P. faberi* Maub. 1, Baarn strain.
- P. faberi* 2, Ashby's strain. This organism has dissociated into variants I, II, and III.
- P. fagi* Hartig, obtained from Baarn.
- P. hibernalis* Carne, American Type Culture Collection.
- P. hydrophila* Curzi, obtained from Holland.
- P. meadii* McRae, obtained from Holland.
- P. melongenae* Saw., obtained from Baarn.
- P. mexicana* Hotson and Hartge, obtained from Hotson.
- P. nicotianae* Breda de Haan. 1, obtained from Baarn.
- P. nicotianae* 2, isolated by Tucker from tobacco in Porto Rico. This organism has dissociated into variants 2-I and 2-II. The writer doubts whether this organism resembles *P. nicotianae*; it is listed here only tentatively.
- P. paeoniae* Cooper, obtained from M. W. Gardner.
- P. palmivora* Butl. 1, obtained from Baarn.
- P. palmivora* 2, isolated by Tucker from *Cocos nucifera* in Porto Rico.
- P. palmivora* 3, isolated by Tucker from *Sabal causiarum* in Porto Rico.

- P. parasitica* Dast. 1. Baarn strain.
P. parasitica 2, isolated by the writer from diseased tomato plants in Morgantown.
P. parasitica 3, Dastur's original strain.
P. parasitica rhei Godfrey, obtained from Godfrey. This organism has dissociated into variants I, II, III, IV, V, VI, VII, VIII, IX, X, XI, XII, and XIII.
P. pini Leon., isolated by R. G. Pierce from roots of *Pinus resinosa* and sent to the writer by Annie R. Gravatt.
P. richardiae Buis., obtained from Holland.

The following thirty organisms were sent by Carl Hartley and represent his tropical Phytophthoras. Detailed information about them may be obtained in a paper by the writer (Physiological studies on the genus *Phytophthora*, Amer. Jour. Bot. 7: 446, 1925). These organisms are listed under Hartley's original numbers as follows: 102, 120, 123, 126, 127, 132 (this organism has dissociated into variants I and II), 136, 137, 138, 139, 143 (this organism has dissociated into variants I and II), 144, 145 (this organism has dissociated into variants I and II), 22, 26, 97, 100, 102, 116, 117, 141, 8, 36, 44, 121 and 130. With the exception of the last five organisms, which should be placed in two or possibly three new species, these all belong to the *P. omnivora* group; the best known and the commonest members of this group have been known as *P. parasitica*, *P. faberi*, and *P. palmivora*.

TECHNIC

One nutrient agar and one nutrient solution were used throughout the work. The nutrient agar contains the following constituents:

| | | |
|-------------------------------------|------|-------------------|
| Dry malt extract..... | 5 | grams |
| Dihydrogen potassium phosphate..... | 0.5 | " |
| Magnesium sulfate..... | 0.5 | " |
| Agar-agar..... | 20 | " |
| Distilled water..... | 1000 | cubic centimeters |

The nutrient solution consists of the following ingredients:

| | | |
|-------------------------------------|------|-------------------|
| Proteose peptone..... | 2 | grams |
| Dihydrogen potassium phosphate..... | 0.5 | " |
| Magnesium sulfate..... | 0.5 | " |
| Succinic acid..... | 0.2 | " |
| Dextrose..... | 5 | " |
| Distilled water..... | 1000 | cubic centimeters |

This solution induces an excellent growth in nearly all Phytophthoras, a slowly growing organism like *P. phaseoli* not excepted.

The solution was divided into five series. Enough malachite green was added to each series to give one part of the dye to sixteen million, eight million, four million, two million, and one million parts of nutrient solution, respectively. These were then tubed by pouring five cubic centimeters of the solution in each test tube and sterilized at 10 pounds pressure for 15 minutes.

The nutrient agar was poured in petri dishes, at the rate of 15 cubic centimeters in each, and sterilized at 10 pounds pressure for 15 minutes.

The *Phytophthora* cultures were then transferred to the plates and were allowed to grow at 25° C. After a large enough colony was formed, inoculum-discs were cut all along the outermost edge of the colony by means of a cork borer having a bore of six millimeters. These inoculum-discs were then transferred to the variously treated solutions and kept at 25° C. for two weeks; at the end of this time the readings were made.

The work has been repeated at least once; where responses have been decidedly erratic, the experiment has been repeated several times.

RESULTS

Arranged according to their growth reactions the following groups of *Phytophthora* are obtained:

Group 1. No growth in presence of one part of malachite green to sixteen million parts of nutrient solution.

P. colocasiae
P. richardiae
P. sp. 26

Group 1a. Only sporadic growth in presence of one part of malachite green to sixteen million parts of nutrient solution.

P. cactorum 12 *P. faberi* 2-I
P. cactorum 24 *P. faberi* 2-II
P. cambivora *P. parasitica rhei* III
P. cryptogea *P. sp. 100*

Group 2. No growth in presence of one part of malachite green to eight million parts of nutrient solution.

P. cactorum 1
P. cactorum 3
P. cactorum 4
P. cactorum 8
P. cactorum 9
P. cactorum 24
P. cambivora
P. cryptogea
P. colocasiae
P. erythrosepatica
P. faberi 2-I
P. faberi 2-II
P. fagi
P. paeoniae
P. palmivora 1
P. palmivora 2
P. parasitica rhei XIII
P. pini
P. richardiae
P. sp. 123
P. sp. 26

Group 2a. Only sporadic growth in presence of one part of malachite green to eight million parts of nutrient solution.

P. boehmeriae
P. cactorum 2
P. cactorum 6
P. cactorum 7
P. cactorum 11
P. cactorum 12
P. cactorum 13
P. cactorum 14
P. cactorum 16
P. cactorum 19
P. cactorum 21
P. cactorum 23
P. citricolum
P. faberi 1
P. nicotianae 2-I
P. palmivora 3
P. parasitica rhei III
P. parasitica rhei VII
P. parasitica rhei XII
P. sp. 102
P. sp. 126
P. sp. 132-II
P. sp. 137
P. sp. 142-II

Group 2b. Growth in presence of one part of malachite green to eight million parts of nutrient solution.

P. cactorum 5
P. cactorum 20
P. capsici
P. cinnamomi 1
P. cinnamomi 2
P. citrophthora
P. faberi 2-III
P. hibernalis
P. hydrophila
P. meadii
P. melongenae
P. mexicana
P. nicotianae 1
P. nicotianae 2-II
P. parasitica 1
P. parasitica 2
P. parasitica 3
P. parasitica rhei I
P. parasitica rhei II
P. parasitica rhei IV
P. parasitica rhei V
P. parasitica rhei VI
P. parasitica rhei VIII
P. parasitica rhei IX

| | |
|-------------------|------------------------------|
| <i>P. sp. 22</i> | <i>P. parasitica rhei X</i> |
| <i>P. sp. 97</i> | <i>P. parasitica rhei XI</i> |
| <i>P. sp. 100</i> | <i>P. sp. 120</i> |
| <i>P. sp. 117</i> | <i>P. sp. 127</i> |
| <i>P. sp. 141</i> | <i>P. sp. 132-I</i> |
| | <i>P. sp. 136</i> |
| | <i>P. sp. 138</i> |
| | <i>P. sp. 139</i> |
| | <i>P. sp. 142-I</i> |
| | <i>P. sp. 143</i> |
| | <i>P. sp. 144</i> |
| | <i>P. sp. 145-I</i> |
| | <i>P. sp. 145-II</i> |
| | <i>P. sp. 116</i> |
| | <i>P. sp. 8</i> |
| | <i>P. sp. 36</i> |
| | <i>P. sp. 44</i> |
| | <i>P. sp. 121</i> |
| | <i>P. sp. 130</i> |

Group 3. Only sporadic growth in presence of one part of malachite green to four million parts of nutrient solution.

P. boehmeriae
P. cactorum 19
P. cinnamomi 1
P. cinnamomi 2
P. faberi 2-III
P. nicotianae 2-I
P. parasitica 2
P. parasitica rhei VIII
P. parasitica rhei X
P. sp. 102
P. sp. 127
P. sp. 136
P. sp. 142-I
P. sp. 143
P. sp. 144
P. sp. 145-I
P. sp. 97
P. sp. 36

Group 3a. Growth in presence of one part of malachite green to four million parts of nutrient solution.

P. cactorum 5
P. capsici
P. hibernalis
P. hydrophila
P. melongenae
P. mexicana
P. parasitica 3
P. parasitica rhei II
P. parasitica rhei IV
P. parasitica rhei V
P. parasitica rhei IX
P. sp. 120
P. sp. 132-I
P. sp. 139
P. sp. 145-II
P. sp. 44
P. sp. 121
P. sp. 130

Group 4. Only sporadic growth in presence of one part of malachite green to two million parts of nutrient solution.

P. cactorum 5
P. capsici
P. mexicana
P. parasitica 2
P. parasitica rhei II
P. parasitica rhei V
P. sp. 132-I

Group 4a. Growth in presence of one part of malachite green to two million parts of nutrient solution.

P. hydrophila
P. melongenae
P. sp. 139

P. sp. 143
P. sp. 145-II
P. sp. 44
P. sp. 121
P. sp. 130

Group 5. Slight and sporadic growth in presence of one part of malachite green to one million parts of water.

P. hydrophila

Perhaps the most outstanding fact brought out by the foregoing table is the extreme sensitiveness of *Phytophthora* to malachite green. It is also interesting to note that nearly all strains of *P. cactorum*, including *P. fagi* and *P. paeoniae*, fail to grow in the presence of one part of malachite green in four million parts of the nutrient solution. *P. cactorum* 5 shows a sharp deviation in this respect and makes some growth even when the proportion of dye and nutrient solution stands at one part to two million, while *P. cactorum* 19 only once made some growth in the one to four million solution. Of the three variants of *P. faberi*, 2-III grows in one to four million solution, while 2-I and 2-II make only a slight and sporadic growth even in the presence of one part of malachite green in sixteen million parts of the nutrient solution. The thirteen variants of *P. parasitica rhei* are by no means all alike in their reactions toward malachite green, as the foregoing table reveals.

DISCUSSION

Too many borderline reactions preclude sharp specific differentiations. It is doubtful if very many clear-cut separations can ever be established within the species of a given genus or even within the strains of a given species; the greater the number of strains studied, the greater will be the individual variations. Consequently, it is a mistake to consider the species as a unit; sometimes it seems that even a single-spore culture is no longer a unit but a complex entity composed of indefinite numbers of units. The numerous dissociations encountered in this genus lead to no other conclusion. At first glance this seems to lead to a hopeless situation; indeed, if we were to attempt the classification of the genus *Phytophthora* on purely genetic lines, such a gloomy outlook would be inevitable. But there is a distinct trend away from genetic classifications towards a more or less artificial segregation of the genus. After all, until we are able to synthesize the species, phylogeny will have no more than a theoretical value. Since taxonomy is primarily for our own convenience, a broader and more tolerant treatment of the genus is certain to yield better results. Consequently, the species concept should be formed upon the average of the behavior and morphology of a majority of the strains. For instance, since most of the strains of *P. cactorum* cannot tolerate one part of malachite green in four million parts of nutrient solution, it is comparatively safe to put down this concentration of the dye as the specific limit for *P. cactorum*. Similarly, in

this species the antheridia may sometimes be amphigynous, but because they are more preponderatingly paragynous, it is safe to assume that *P. cactorum* is specified by paragynous antheridia. The next group of which there are a large number of strains is *P. omnivora*, which is composed of *P. parasitica*, *P. palmivora*, *P. faberi*, *P. colocasiae*, *P. nicotianae* and perhaps a number of others. Because this is the most widespread and the most cosmopolitan of all Phytophthoras, it is decidedly more variable and complex than *P. cactorum*; consequently, where perhaps some twenty or thirty strains would suffice to trace the behavior average of *P. cactorum*, at least one hundred strains would be necessary for a similar treatment of *P. omnivora*; a difficult but decidedly valuable task. Judging from the reactions of the strains in the foregoing table, it appears that the critical concentration of malachite green for *P. omnivora* would be somewhere between one to four million and one to two million parts of nutrient solution, whereas that for *P. cactorum* is in the neighborhood of one to four million.

Nothing definite can be said concerning the remaining species, unless more strains become available. They merely indicate that malachite green is well adapted to studies on differential growth with *Phytophthora* and should find a wider use with investigators.

SUMMARY

1. Malachite green, at the rate of one part of the dye to 1, 2, 4, 8, and 16 million parts of nutrient solution, was used to induce differential growth in *Phytophthora*.

2. Three organisms failed to grow when malachite green was present at the rate of one part of the dye to 16 million parts of nutrient solution, and eight others made only a sporadic growth. Twenty-one organisms failed to grow when the amount of the dye was doubled, and twenty-nine others made only a sporadic growth. Only one organism was able to make a growth, very poor and sporadic, in the presence of one part of malachite green in one million parts of nutrient solution.

3. The critical concentration of the dye for the *P. cactorum* group is in the neighborhood of one part of malachite green in four million parts of nutrient solution, while that for *P. omnivora* is decidedly higher. While the remaining species, because of an insufficient number of strains, cannot be properly grouped according to their reaction toward malachite green, yet they clearly indicate that this dye may serve admirably in defining certain groups of more or less closely related strains or species.

THE MORPHOLOGY AND ANATOMY OF THE INFLORESCENCE AND FLOWER OF THE PLATANACEAE

LUCY E. BOOTHROYD

(Received for publication March 23, 1930)

The fundamental nature of the flower and inflorescence of the Platanaceae has been very obscure because of the extremely compressed nature of the latter. Some texts have figured and described the flower as having normal perianth parts; others state that it entirely lacks a perianth. The kind of inflorescence and especially the type of flower play an important rôle in determining relationships between groups of plants. Consequently, uncertainty concerning these structures has led to much difference of opinion regarding the affinities of this family. The morphology and vascular anatomy of these parts have been studied previously, but further study, especially of the vascular system, has seemed advisable in order that a clearer conception of the nature of the inflorescence might be gained.

GENERAL DESCRIPTION

Platanus, the single genus of the Platanaceae, consists of three or four species, all large trees with a characteristic exfoliation of the outer bark. The species are *P. orientalis* L. of Europe and Asia Minor, *P. occidentalis* L. found in the eastern part of the United States, and *P. racemosa* Nutt. of the southwestern United States and Mexico. *P. acerifolium* Willd. is another rather generally recognized species but the writer agrees with L. H. Bailey (1) that it is only a hybrid between *P. orientalis* and *P. occidentalis*.

The leaf of *Platanus* has three to seven lobes similar to those of *Acer* and *Liquidambar*, with the largest number of lobes in the leaf of *P. orientalis*, and three commonly in *P. racemosa*. The margin is dentate in all forms except *P. racemosa*, which commonly has entire lobes. In all species the petiole has a circular concavity at the base, which covers the bud. The large leafy stipules encircle the twig.

The inflorescence consists of from one to several globular, unisexual heads of closely arranged flowers distributed on an elongate pendent peduncle. Each head has a circular bract at its base, and also thin leaf-like bractlets among the flowers. *P. occidentalis* has only one head on each peduncle, except in rare cases when there may be a small second head at the base of the large one. *P. acerifolium* has from one to three, mostly two, almost sessile heads. *P. racemosa* may have from two to five, but has more generally three or four balls on a peduncle. In this species the

lateral heads are often stalked with a peduncle one centimeter long. According to Bailey (1) *P. orientalis* has three or more heads on the peduncle.

THE FLOWER

The flowers of *Platanus* have been inadequately and more or less inaccurately described in texts up to the present time. In fact, the writer has been unable to find any figures or good descriptions of the flowers of *P. racemosa*. In all species the flowers are unisexual (except in rare cases), with their members in whorls. The pistillate flower of the species studied is strictly hypogynous. The free pistils are arranged in two or three whorls of three or four each, and vary in number from five to nine per flower (figs. 1, 2). In most cases where five or seven pistils are present the position of the outer floral organs indicates the loss of a carpel. This loss generally occurs in the inner whorl of carpels. Abnormal and partially aborted carpels are common in the inner carpel whorl of pistillate flowers. A whorl of three or four staminodia surrounds the pistils, its members alternating with them.

The above characteristics are common to the pistillate flowers of all species, but the perianth parts vary considerably. In *P. acerifolium* and *P. occidentalis* (Pl. XL, fig. 1) there is a well-defined cup-shaped structure, two to three cells thick, surrounding the base of staminodia and pistils (as noted by Griggs, 16). This cup, which apparently represents the calyx, is shallowly lobed and covered with hairs on the margin. In *P. racemosa* (fig. 2) distinct sepals are found in place of this cup. These are as long as the staminodia and are free at the base.

In pistillate flowers petals were found only in *P. acerifolium*. These organs vary much in size, but when present are sufficiently large to be easily distinguished under the dissecting microscope.

The staminate flowers are more regular in form and the number of flower parts is more definite than in the pistillate flowers. There is in all species one whorl of from three to five stamens. Petals are present alternating with the stamens. A calyx cup similar to that of the pistillate flower exists in *P. occidentalis* and *P. acerifolium* but is lacking in *P. racemosa* (figs. 3, 4). Staminate heads of all species may have occasional rudimentary carpels in a few of the flowers of the head.

THE FLORAL PARTS

The pistil consists of one simple carpel. This is long and slender and is incompletely closed, being open near the top of the ovary (fig. 1). The ovary is small and the single loculus is filled by the one or two ovules except for an elongate cavity in the upper part, which opens to the exterior just below the stigma. The stigma extends all along the inner face of the long slender style almost as far as the ovary. The style and stigma are persistent and closely resemble those of *Liquidambar* and *Fothergilla* of the Hamamelidaceae.

The pistil is follicle-like in its general structure. The one or two ovules are borne on the margins as in a typical follicle, and often abortive or vestigial ovules occur in the loculus above the normal ovules, also borne on the margins. This suggests that the carpel is essentially primitive in this genus.

The vascular anatomy further emphasizes this. Though the pistil is very small and obviously modified in connection with wind pollination (a long stigmatic area and one or two ovules only), it has the vascular supply of a follicle with several well-developed ovules. Three traces supply the carpel, a dorsal and two ventrals, arising separately from the receptacle. The dorsal bundle gives off two lateral branches at the very base of the carpel (fig. 11), and runs on to the tip of the stigma (figs. 10, 11-17). The branches pass up the sides, parallel with the dorsal, and dwindle out near the top of the ovary. The two ventrals pass up, also parallel with the dorsal, and dwindle out in the base of the style. The ovule is supplied by one of the two ventral bundles (fig. 14). If there are two ovules, each of the ventrals supplies one of them (figs. 18-20). Often only one fertile ovule is present in the loculus but a second reduced one can also be found. The ovule trace to the latter can often be found, and is generally derived a little higher than that of the first ovule (fig. 22). This position may be accidental. On the other hand, it may indicate that reduction of the upper ovules of a primitive follicle (leaving only one or two basal ones) is responsible for the type of carpel found in *Platanus*. The anatomy is thus definitely that of the primitive carpel. The pistil is therefore in form and structure distinctly primitive, though reduced and modified in some ways.

The stamen has a very short filament and long anthers with a rather large connective, which expands at the top into a large fleshy cap covering the anthers. These caps are covered with short hairs, and until anthesis fit closely together making a tight covering over the whole ball. A single vascular strand runs up through the filament and connective into this cap where it branches slightly and disappears. Griggs (16) and Clark (12) have suggested that this stamen is very similar in form to the microsporophyll of the Abietineae with its distal enlargement. This similarity is probably due to parallel development and related to the protection of the pollen until shedding in both cases. *Platanus* recalls the Abietineae in no respect except the "orthotropous" ovules. The anthers are large and contain much pollen, as might be expected in a wind-pollinated plant.

The staminodia vary from slightly abnormal stamens to irregular fleshy masses. Generally, however, they are about one-fifth the size of the normal stamen with a short slender filament and an upper fleshy part, suggesting the cap and shortened anthers. The apex is covered with short hairs, and a single vascular bundle runs up through the center as in the normal stamen. The staminodia are clearly sterile stamens.

The parts of the flower commonly believed to be petals are the most variable. In the staminate flowers of *P. occidentalis* and *P. acerifolium* they are generally fleshy, three-pronged structures with the prongs fitting closely between the bases of the stamens (fig. 3). They are sometimes slightly fused at the base in these forms. In *P. racemosa*, the petals of the staminate flower are reduced to tiny rounded knobs (fig. 4). The pistillate flowers of *P. acerifolium*, however, have strap-like petals, which vary greatly in length, the longest being about one-fifth the length of the pistil. In all species the petals can be distinguished from the other parts of the flower by the absence of hairs and vascular tissue (a fact noted by Bretzler, 5). In *P. occidentalis* and *P. acerifolium* they somewhat resemble the staminodia of pistillate flowers. There are, occasionally, unconnected xylem strands beneath the base of the petals but these have never been observed to enter the petal itself.

The petals in this genus are therefore clearly vestigial organs, ranging from fairly petal-like strap-shaped structures to minute irregular bodies. The presence of vestigial traces to some of these organs is further proof that they are not merely glands. The three-lobed condition in the forms which have these least reduced suggests that the ancestral petal was possibly three lobed.

The calyx is also strongly reduced, and ranges from a whorl of very small but distinctly sepal-like structures, in *P. acerifolium*, to a mere shallow cup with lobes. Where the sepals are free and obvious (fig. 2), their shape distinctly suggests the bracts of this family. No trace of vascular supply to the calyx was found.

The flowers of *Platanus* are in many respects like those of other wind-pollinated Angiosperms, especially those of the Amentiferae. Though they are of primitive type, they are markedly specialized by reduction in adaptation to wind pollination, and by the condensation of the inflorescence. The numerous pistils, somewhat indefinite in number, free, hypogynous, and follicle-like, are abundant evidence of primitiveness. The presence of vestigial stamens (staminodia) in the pistillate flowers, and of vestigial pistils in the staminate flowers is strong evidence that these flowers were once perfect. The presence of a fairly well-defined corolla and calyx shows that a perianth of two whorls formerly was possessed by these flowers.

The pistillate flowers are more primitive in their somewhat unsettled condition as to number of parts. The staminate flowers, however, though with but one whorl of sporophylls with rather definite numbers, retain more markedly a corolla.

THE ANATOMY OF THE INFLORESCENCE

As has already been mentioned the number of heads in an inflorescence varies from five or six to one. The condition where there are several on the peduncle is probably more primitive than that where there is but one,

as will be shown below. A description of the anatomy of the main peduncle of *P. racemosa*, a species with several heads, will serve as an example of the primitive and less reduced condition of the inflorescence. Below the insertion of the first head the vascular system of the peduncle consists of two concentric cylinders of bundles. The segments of the outer cylinder have the phloem external to the xylem while the inner group of bundles show the opposite orientation. The bundles of the inner group are derived by lateral splitting from the bundles of the outer cylinder in the lower part of the peduncle (Pl. XLI, fig. 24), a condition also observed by Bretzler (5). This division of the vascular tissue into two cylinders probably has no significance except a mechanical one.

At the level of insertion of the first head, five bundles pass out, becoming a circle of bundles and leaving a single large gap (figs. 25-26). The supply to the remaining lateral heads is similar (figs. 27-30).

The head, which appears to be terminal on the axis, has one of two types of supply. Either the entire set of bundles remaining in the peduncle passes directly into it and supplies the various parts, or some of the bundles may extend up the side of the ball a short distance and end without giving off branches to the head itself (fig. 31). The latter situation often occurs where less than five heads are present on the peduncle but has not been observed when five or six are present. It appears that sometimes the terminal head is truly terminal; in other cases it is falsely so, and the bundles running up the side of the ball and dying out represent the continuation of the peduncle vestigially beyond the head. This terminal remnant of the peduncle is appressed to the side of the ball and merged with it. This would seem to indicate that five heads on the peduncle is a relatively primitive condition in this species, and that when four or less are present, the terminal portion of the peduncle is represented by only a remnant of vascular tissue.

Further evidence of this terminal reduction in the number of heads on the peduncle is to be had in *P. acerifolium*. When this species has a fruiting inflorescence of only two or three balls there is often beyond the last fruiting head a projection of the axis tipped with a small hairy knob, and a second similar hairy knob may even occur in the position of a lateral head. These appear to be vestigial heads.

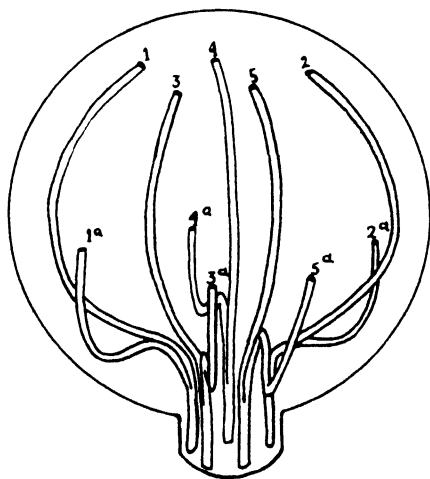
Platanus occidentalis has but one head as compared with several in other species. A difficulty in the way of explaining a reduction of the five or six heads of *P. racemosa* to the single head of *P. occidentalis* is that the latter species shows no signs of such reduction except the presence near the base of the head of an occasional very small strand which supplies no organs. That reduction of this type takes place in *P. acerifolium* and *P. racemosa* is so obvious, however, that one can safely infer that it has taken place in the evolution of the inflorescence of *P. occidentalis*, which is thus the most specialized species in the genus as to its inflorescence.

The position of the gaps as well as the balls indicates a two-fifths arrangement of these branches of the inflorescence.

The anatomy of the head is complex because of the large number of florets (95-125) crowded on a small globular receptacle. The vascular supply of the head is essentially the same for all three species studied, and can best be followed by means of a series of transverse sections beginning at the base of the head and extending to the apex.

The five bundles already mentioned enter the base of the head in a ring, and a strong lateral branch is soon given off from each. The ten bundles so formed pass into the ball unchanged for some distance (figs. 32-33).

At the base of the head five distinct groups of florets are distinguishable. These five groups are supplied by the five branches of the original bundles of the ball, which descend to the flowers. Anatomically as well as externally



TEXT FIG. 1. Diagram of a *Platanus* head showing the five major bundles to the head, 1, 2, 3, 4, 5 respectively, and their major branches 1a, 2a, 3a, 4a, 5a, respectively.

the groups are distinct because of the broad parenchymatous segments of the "receptacle" between them (fig. 33). The arrangement of the vascular supply of each of these flower groups, taken as whole, strongly suggests a much telescoped branch of an inflorescence.

These downward branches to the lowermost flower clusters, and also the flower clusters themselves, are clearly arranged in a flattened two-fifths spiral (figs. 32-34). The order of their attachment to the main bundles of the cylinder of the ball is that of a two-fifths arrangement.

After the departure of the strands to the lower tier of clusters, the five main bundles pass up and out between these groups sending off lateral branches supplying another group of flowers which alternate with the five basal groups and form the upper part of the ball. These five bundles are distinguishable to the top of the ball (figs. 34-37) where they finally break

up entirely to form the branches to the flowers at the apex. There are thus ten regions of the ball, five at the base and five alternating with these in the upper part.

It is evident that each ball is a much compressed inflorescence branch, fundamentally of five parts, each part with two sections (a lower branch and an upper terminal portion, text fig. 1) making ten sections. Each of the five main branches is arranged according to the two-fifths plan common to the branches and leaves of *Platanus*. Each segment (tenth) of the inflorescence bears several to many flowers.

The peduncle bears typically three to five balls, and these are also arranged according to the two-fifths phyllotaxy. An elongate, highly complex inflorescence has apparently been condensed, each of the major branches being condensed by the fusion of its parts into a ball. The terminal portion has similarly formed a ball. In the more advanced species the number of these has been reduced until, where but one is present, only the basal persists.

According to Brouwer (9) there are ten morphological and anatomical regions of the head, five of the lower part and five higher up alternating with these. She states that these are distinguishable in the fruiting head by the length of the fruits in each region, those on the periphery of a region being the shortest and those of the center the longest. Each of these regions is supplied by one of the ten bundles present at the base of the ball. With this statement the writer agrees so far as the morphological and anatomical situation is concerned.

Remnants of vascular tissue are sometimes found supplying the bracts. The circular bract at the base of the ball may have two or three bundles entering it but these are so fragmentary that their origin could not be determined. Occasionally one of the bractlets among the flowers may have a weak vascular bundle entering it. This, also, is very much reduced and can be followed only a short distance. It is quite clear from these remnants of vascular supply that the bract and bractlets are reduced structures.

The vascular supply of the flowers is relatively simple. The bundles enter the flower base in a ring, and in a short distance pass out to the parts which they supply.

The vascular system of the pistillate flower is the more complex on account of the greater number of bundles. For the sake of simplicity a pistillate flower of six carpels is figured and described. When three whorls of carpels are present in the flower the situation is the same except that there is an additional whorl of bundles inside the inner whorl here described. A ring of bundles equal in number to the carpels of the flower enter its base (Pl. XL, fig. 9). First the bundles to the staminodia are given off tangentially from alternate carpel bundles (fig. 8), the latter simultaneously moving to a position slightly inside the remaining bundles. At the same time two ventral carpel bundles split off laterally from each dorsal

bundle (fig. 8) and swing around into a position radially in line with the dorsal (fig. 7). At this level the carpels are separate and the lateral bundles are given off from the dorsal and swing around into place half way between the ventrals and the dorsal (fig. 6).

The bundle supply of the staminate flower consists simply of three to five bundles passing directly out into stamens with occasionally small remnants of bundles between these under the bases of the petals.

The anatomy clearly bears out the usual interpretation of the flower, namely that it is composed of alternating whorls of floral organs. The vascular supply of each such flower forms a separate cylinder, which is distinct from those of other flowers. The staminodia bundles are tangentially fused with the bundles of the carpels in the whorl opposite them (the inner carpel whorl when only two whorls of carpels are present). The remaining carpels have separate bundles which are not fused with those of any other whorl. The bundle supply of each stamen is also free from other bundles. Petals lack vascular supply except in the staminate flower where they may have fragments of vascular tissue beneath them. The much reduced calyx entirely lacks vascular strands. The anatomy as well as the morphology of the *Platanus* flower thus shows evidence of original simplicity (parts free in alternating whorls) and subsequent reduction (indicated by remnants of vascular tissue to the petals and lack of vascular supply to the calyx).

DISCUSSION

The previous morphological studies of *Platanus* have, for the most part, been attempts to interpret the gross structure of the flower. The first of these was undertaken by Clark (12) in 1858, who discovered the presence of the petals, sepals, and bracts in *P. orientalis*. Judging by his figures the relation between the sepals and bracts was not clear to him.

Schönland (34) in 1883 and Bretzler (5) in 1924, both investigated the morphology and ontogeny of the flower and fruit of *P. orientalis* and *P. acerifolium*, and were of the same opinion fundamentally in respect to the general structure. Both found much reduced petals and sepals. Schönland, however, in the pistillate flower shows double the number of parts in each whorl of staminodia, petals, and sepals, making these organs always equal in number to the pistils. Both Bretzler (5) and Brouwer (8) corrected this error. Moreover, Schönland and Bretzler describe the pistillate flowers as being perigynous instead of hypogynous as determined by Griggs and the writer. Bretzler, as has been mentioned, also described the general anatomical situation in the peduncle. She seems, however, to have made no attempt to follow the course of the bundles in the peduncle.

Griggs (16) in 1909 described the ontogeny of the staminate and pistillate flowers of *P. occidentalis*. He described the calyx cup, which he thinks may be either bract or calyx, and noticed an absence of petals in the pistillate flower. He dismisses the petals of the staminate flower by suggesting that

they may be carpels, an interpretation which is hard to understand when one considers that their insertion is outside the whorl of stamens. This led him to conclude that the flower of the Platanaceae was a much simpler thing than that described by Schönland and that it lacks a corolla. Moreover, he was unable to determine a symmetrical arrangement of the parts. On the basis of his studies he placed the Platanaceae with the Urticales.

However, structures suggesting petals in their position are present in the pistillate flowers of *P. acerifolium* and *P. orientale* although their form is variable, and petals are present in the staminate flowers of all species. By a comparison of the so-called calyx of *P. occidentalis*, *P. acerifolium*, and *P. racemosa* it is clear that this structure may consist of either fused or entirely free sepals in different species.

The interpretation to be placed on the structures which have been called petals and sepals, has, however, not been perfectly clear to the present writer. Whether such minute structures, lacking vascular tissue, can rightly be called perianth parts is perhaps questionable. Extreme reduction due to compression, however, may well have brought about this condition, as is suggested by the rudimentary bundles to some petals. Moreover, it is difficult to find another interpretation of these organs which stand in the position for petals and sepals.

Brouwer (8, 9) studied the anatomy of the inflorescence, and interprets the flower in a way very different from that of any other writer including the present one. A *Platanus* flower, according to her interpretation, consists of a pair of organs only, a pistil (or "fertile leaf"), and a scale, which has become fertile secondarily, becoming thus a stamen. The former suggests a fertile scale of the ovulate cone of a conifer, and the latter its subtending bract. Thus in her own words (8): "The ball . . . has some resemblance to the conus of gymnosperms." She states that the calyx is only to be regarded as a proliferation of the ball. The so-called petals, in her estimation, are staminodia, or infertile scales subtending fertile carpel leaves. The presence of unisexual balls is the result of reduction, at least in the case of the staminate ones.

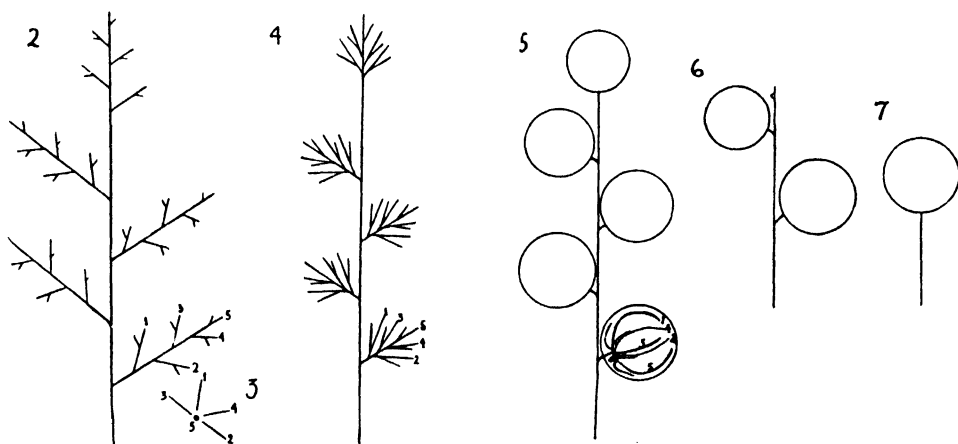
Her evidence lies chiefly in the vascular anatomy. In a longitudinal section of the flower a common bundle is often seen to supply a pistil and a staminodium or stamen. This, to her, is proof that these two structures form one flower. But this is a common and quite normal situation in angiosperms whenever a staminodium or stamen stands opposite a pistil in the flower. She fails to note, however, the very obvious fact that the bundles to the group of organs commonly called a flower form a distinct cylinder running for some distance into the receptacle (figs. 6-9).

As evidence that the stamen is only secondarily a fertile organ, being primarily a scale corresponding to the bract of gymnosperm cones, she cites the case of the pistillate head, which entirely lacks fertile stamens, and the protective nature of the cap of the staminodia in the young pistillate

head. However, the entire lack of fertile stamens in the pistillate flowers is just as logically explained by reduction as is the complete absence of carpels in the staminate head, and the protective nature of the cap in the stamen may be assumed to represent a secondary modification of this organ.

Brouwer's interpretation of the inflorescence is also different from the present one. Since the entire inflorescence arises in ontogeny as a single meristematic projection, and during later growth splits into several heads, she concludes that in the primitive condition there is only one head, and that the growing out of the peduncle between the parts of this head in ontogeny divides it into several false groups. This explanation of the condition just described seems without value since the terminal meristematic portion of any axis, whether it is about to form a terminal vegetative branch system, a single flower, or a branch system of flowers, commonly arises as a single projection.

As a result of the present anatomical investigation it seems to the writer that the inflorescence as a whole is racemose. The primitive form may well have been a panicle. The major branches of such an inflorescence have been reduced, and their parts ultimately fused into heads. The



TEXT FIGS. 2-7. Diagrams to show the evolution of the *Platanus* inflorescence. FIG. 2. Compound raceme. FIG. 3. Diagram to show the arrangement of the parts of one of the major branches of the inflorescence. FIG. 4. Diagram to show the reduction of the major branches of the compound raceme. FIG. 5. Diagram to show the formation of heads (by the fusing of branches); 1, 2, 3, 4, 5 correspond to the parts represented by those numbers in FIGS. 2-4, and each represents a flower branch bearing from 15-20 flowers. FIGS. 6, 7. Diagrams to represent the terminal reduction of the inflorescence resulting in the single head found in *P. occidentalis*.

terminal portion has likewise formed a head. The further modification of the inflorescence has involved the loss of the distal portions, and in the extreme case of reduction (*P. occidentalis*) only the basal ball persists (text figs. 2-7).

TAXONOMIC POSITION OF THE PLATANACEAE

During its taxonomic history *Platanus* has been placed with many different dicotyledonous groups, and has even been considered as a connecting link between Angiosperms and Gymnosperms. Only the more generally accepted tendencies in regard to the affinities will be discussed. These fall into two groups. Some authors have assigned *Platanus* to the Urticales. Others have placed it near the Rosaceae and Hamamelidaceae.

The idea of a close affinity with the Urticales was more generally held among earlier writers. Lindley (25) first suggested this alliance as preferable to the older idea of a mental relationship. He included the Artocarpeae and the Liquidambarae in the Urticales and considered *Platanus* the connecting link between the two but more closely related to *Artocarpus* because of the simplicity of the carpel.

Van Tieghem (36) follows Lindley's classification in this respect, but omits *Liquidambar* from the Urticales, placing it instead in the Hamamelidaceae to which Hooker had previously referred it, and where modern classifications retain it.

Eichler (14) related *Platanus* to *Artocarpus* in the Urticales on the type of inflorescence and stipule.

Finally, in 1909 Griggs (16) studied *P. occidentalis* and again placed the genus near *Artocarpus* (and *Ficus*) largely on account of the irregular arrangement of parts, the reduced perianth of *Platanus*, and the type of inflorescence and stipule.

The evidence for a close affinity between the Urticales and *Platanus* is rather weak. As mentioned above, this relationship depends on the similarity of appearance of the inflorescence and stipules in *Artocarpus* and *Platanus*, the simplicity of the pistil, the irregularity in alternation of whorls, and the inconstancy in number of parts of the flowers. But *Platanus* has a fundamentally regular flower and the pistil of the Urticales is not a simple carpel (3) as in *Platanus*. There seems to be little left to show any very close affinity between the two.

The majority of writers, especially the more recent ones, have favored a closer affinity of *Platanus* to the Rosaceae and Hamamelidaceae than to the Urticales. In 1843 Brongniart (7) placed this genus in the Hamamelidaceae near *Liquidambar* (the Balsamifluae).

Later Baillon (2) described *Platanus* as the most reduced genus of the Saxifragaceae, especially the Liquidambarae, but admitted a close affinity to the Hamamelidaceae.

Schönland (34) considered a possible relationship of the family to the Hamamelidaceae and Saxifragaceae, but on the basis of flower and fruit morphology and distribution concluded that the closest relation is with the Spiraeaceae of the Rosaceae. It is Schönland's figures and ideas, which have been used in many of the more recent treatments of the family, such as those of Engler and Prantl (26) and Engler and Gilg (15). These writers

tend to associate *Platanus* more closely with the Hamamelidaceae. Schönland's figures, however, are somewhat inaccurate, and this has led to serious mistakes in subsequent texts dealing with the Platanaceae. Other students have also called attention to these facts.

The most recent tendency has been to bring the Hamamelidaceae and Urticales nearer to one another. This was expressed by Wettstein (39) who places *Platanus* in the Hamamelidales, and considers both this and the Urticales as primitive groups.

More recently Hutchinson (18) has derived the Urticales through the Hamamelidales from the Rosales and thus brought together all the groups which have been generally related to *Platanus*. The latter he places in the Hamamelidales as a separate family.

On the whole the present tendency is to depart from the older conception that *Platanus* is primitive on account of its poorly developed perianth, and to derive it by reduction from forms which have a well-developed floral envelope.

In view of the fact that the small compact head of *Platanus* is probably derived from a racemose type of compound inflorescence such a position for the genus seems reasonable. Add to this the fact of the frequent abortion of carpels and the presence of remnants of vascular tissue beneath the petals of the staminate flowers, and it seems quite probable that great reduction has occurred and the perianth has been reduced to its present condition.

As has already been mentioned the number of balls in an inflorescence varies from five or six to one. The condition where there are several heads on the peduncle is probably more primitive than that where there is but one, the latter being the result of terminal reduction.

The majority of writers, especially the more recent ones, as has been already mentioned, have placed the Platanaceae in the vicinity of both the Rosaceae and the Hamamelidaceae. *Platanus* resembles the latter family, and especially *Liquidambar* and *Altingia*, in several respects, notably the form of the stigma, some features of ovule structure (5), the occurrence of staminodia in the pistillate flowers, the tendency to formation of heads, the presence of stipules, the leaf form, and the anatomy of the secondary xylem. On the other hand the heads of the Hamamelidaceae seem to have been derived from simple spikes, and as far as the writer can determine compound racemes of the hypothetical type from which the *Platanus* head might have been derived do not occur in the Hamamelidaceae. The most pronounced difference between *Platanus* and this family is the carpel situation. The Hamamelidaceae are uniform in having only two carpels in the flower, these being generally more or less fused to one another and to the surrounding flower parts. The presence of anatropous ovules in all or most of the Hamamelidaceae and of an almost orthotropous ovule in the Platanaceae is another factor in the separation of these two groups.

The members of the Rosaceae most nearly suggesting *Platanus* are the

Spiraeaceae. These are similar to *Platanus* in stem structure (28), the variable number of free carpels, the structure (5) and orthotropous position of the ovule, and the type of fruit, which is a follicle with few to several seeds. Moreover, panicles and corymbs occur in *Spiraea*, and in related genera compression of corymbs into heads occurs. Stipules are also present in the Rosaceae. *Spiraea*, on the other hand, has a perfect perigynous flower (though sometimes but slightly so) while the flower of *Platanus* is clearly hypogynous and seldom perfect.

That *Platanus* belongs somewhere in the vicinity of the rosaceous complex seems to have been clear to most modern taxonomists, and it may well have been derived from some primitive rosaceous type. However, there still remains a considerable gap between the Platanaceae and either the Hamamelidaceae or the Rosaceae.

SUMMARY

1. *Platanus* has actinomorphic, generally unisexual flowers with mostly free parts alternating in whorls of three or four.
2. The pistillate flowers are hypogynous and apocarpous with five to nine carpels in two or three whorls. There are three or four staminodia alternating with the outer whorl of carpels. In all forms a calyx which has fused sepals in *P. occidentalis* but free sepals in *P. orientalis* and *P. racemosa* surrounds the stamens, its parts being opposite to them. Petals alternate with the staminodia in *P. orientalis* and occasionally in the hybrid *P. racemosa*.
3. The pistil is essentially primitive but is modified in some ways. It is fundamentally a follicle, in which only the basal one or two ovules remain. The stigma extends along the inner face of the style to the top of the ovary. There are five vascular bundles in the ovary, a dorsal, two laterals, and two ventrals. Only the dorsal extends to the tip of the style.
4. The staminate flower is regular, and less variable in the number of its parts than the pistillate flower. Except for rare cases (parts in fives) there are three or four stamens surrounded by three or four petals which alternate with them. There is a calyx of four parts which alternate with the petals in all species except *P. racemosa*. In *P. occidentalis* the sepals are fused.
5. The stamen has long anthers and a copious supply of pollen.
6. The perianth parts are much reduced and all (except the petals of the staminate flower) entirely lack any trace of a vascular supply.
7. The inflorescence is fundamentally a panicle. Its main branches have been reduced to form the heads, the end of the inflorescence being also fused to form a head. This compression has led to great reduction of the bracts and flower parts. Further reduction has resulted in the loss of all the heads but the basal one in *P. occidentalis*. That such reduction has taken place is clearly shown by the morphology and anatomy.

8. The modification of the flower and inflorescence are apparently related to an adaptation to wind pollination. The length of the stigma, the amount of pollen produced, the size of the anthers, the general reduction of the perianth, and the strong tendency toward unisexuality of the flowers are all evidences of this adaptation.

9. The taxonomic position of *Platanus* is generally agreed to be somewhere near the more primitive members of the Rosaceae, on the basis of flower and inflorescence structure.

The writer wishes to express her gratitude to Professor Arthur J. Eames for his helpful advice. She is also indebted to Professor Philip A. Munz of Pomona College for the collection of flowering material of *P. racemosa*.

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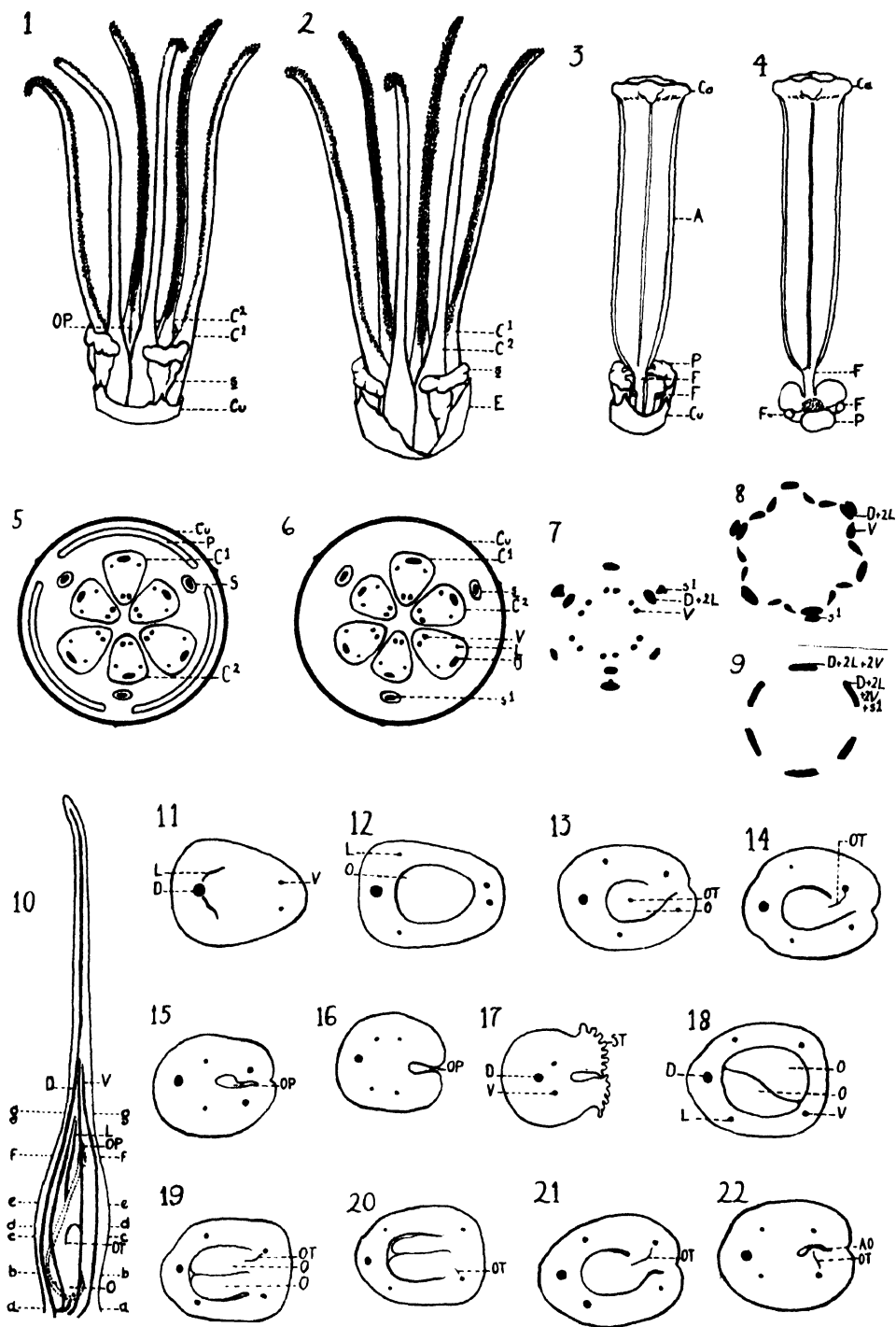
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EXPLANATION OF PLATES

Abbreviations used: *A*, anther; *AO*, abortive ovule; *C*¹, carpel of the outer carpel whorl; *C*², carpel of the inner carpel whorl; *Ca*, cap on the stamen; *Cu*, calyx cup; *D*, dorsal bundle of the carpel; *E*, sepal; *F*, filament of the stamen; *L*, lateral bundle of the carpel; *O*, ovule; *OP*, opening into top of ovary; *OT*, ovule trace; *P*, petal; *S*, stamen; *s*, staminodium; *s*¹, staminodium (or stamen) bundle.

PLATE XL

- FIG. 1. Habit sketch of a pistillate flower of *P. occidentalis* with the hairs removed.
- FIG. 2. Habit sketch of a pistillate flower of *P. racemosa* with the hairs removed.
- FIG. 3. Habit sketch of a staminate flower of *P. occidentalis* with the hairs removed.
- FIG. 4. Habit sketch of a staminate flower of *P. racemosa* with the hairs removed.
- FIG. 5. Diagram of a perfect flower of *P. acerifolium* in transverse section.
- FIG. 6. Diagram of a pistillate flower of *P. occidentalis* in transverse section.
- FIG. 7. Diagram of a transverse section immediately below the insertion of a pistillate flower.
- FIG. 8. Diagram of a transverse section of the same bundle system at a deeper level in the ball.
- FIG. 9. Diagram of a transverse section of the same bundle system showing the six original bundles to such a flower (fig. 6).
- FIG. 10. Diagram to show the vascular bundles of the carpel of *Platanus*.
- FIGS. 11-17. Transverse sections of a carpel with one ovule at levels *a*, *b*, *c*, *d*, *e*, *f*, and *g*, respectively (fig. 8).
- FIG. 18. Transverse section at level *b* of a carpel with two ovules.
- FIG. 19. Transverse section at level of a carpel with two ovules.
- FIG. 20. Transverse section, slightly above level *d* of carpel with two ovules, showing the origin of the ovule trace to the second ovule.
- FIG. 21. Transverse section of a carpel at level *d* with one normal and one abortive ovule.
- FIG. 22. Transverse section slightly above level *d* of a carpel with one normal and one abortive ovule, showing the ovule trace to the abortive ovule.



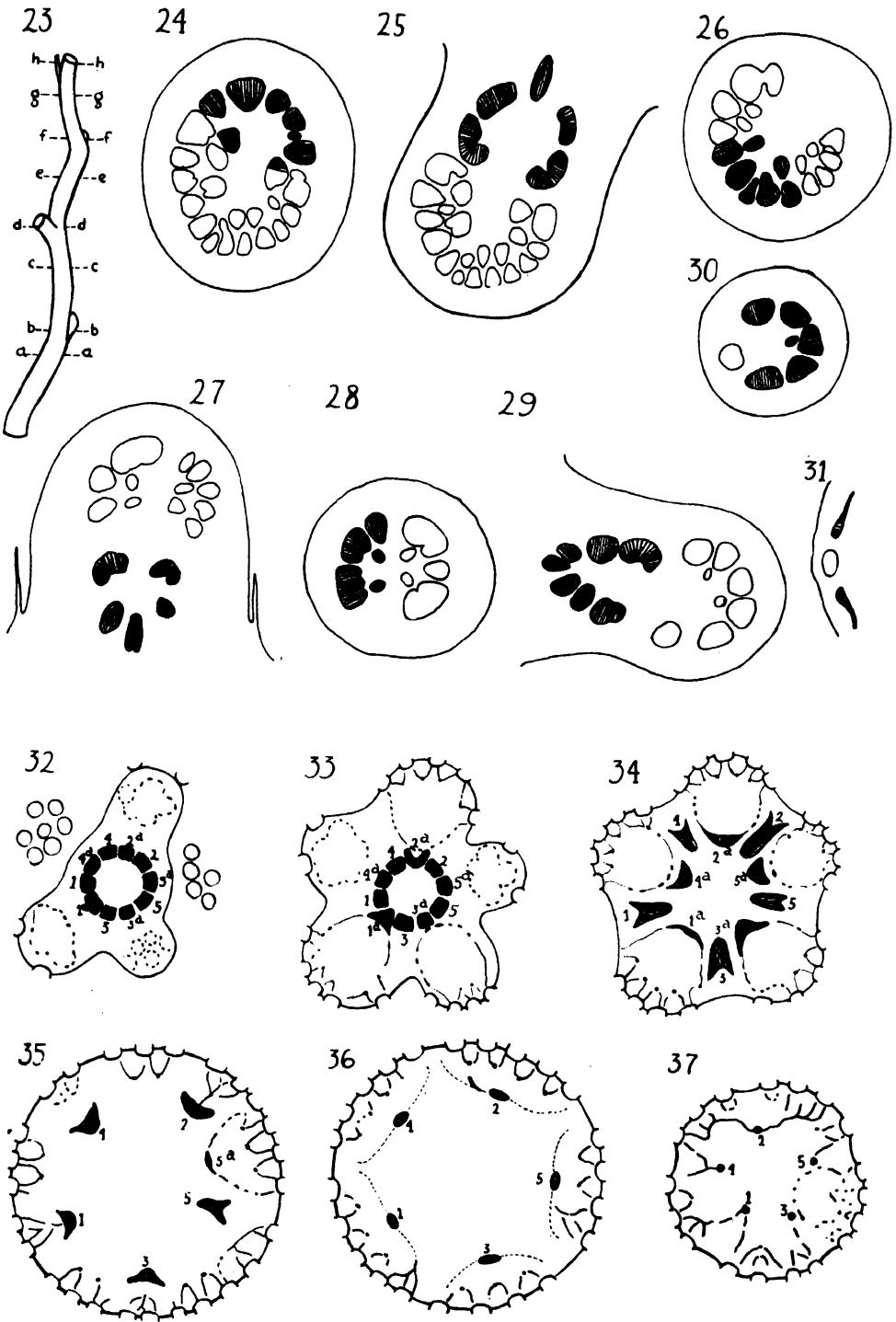


PLATE XLI

FIG. 23. Diagram of the branch system of a peduncle with four heads and an abortive terminal portion (*P. racemosa*).

FIGS. 24-30. Transverse sections at the levels *a, b, c, d, e, f, g*, respectively, of figure 23.

FIG. 31. Transverse section through the side of the pseudo-terminal head of the peduncle showing the remnant of vascular tissue in the side of the head.

FIGS. 32-37. Diagrams of the transverse sections of a head to show the course of the major bundles of the head. 1, 2, 3, 4, 5 are the five bundles to the head. 1*a*, 2*a*, 3*a*, 4*a*, 5*a* are the major branches of these bundles. *o*, a flower in transverse section. *u*, a flower in longitudinal section. *Q*, the bundles to a flower in transverse section.

LOCAL LESIONS ON BEAN LEAVES INOCULATED WITH TOBACCO MOSAIC VIRUS¹

W. C. PRICE

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INTRODUCTION

Necrotic lesions appear at the points of inoculation when tobacco mosaic virus is rubbed over the surfaces of leaves of a number of species of *Nicotiana*. Holmes (1) studied the development of such lesions in five different *Nicotiana* species. Lesions occurring on *N. glutinosa* make their appearance the second day after inoculation and are well developed on the fourth or fifth day. Since their number is largely determined by the concentration of virus in the inoculum, the lesions have been made the basis of a method for the rapid determination of virus concentration.

The purpose of this paper is to describe briefly similar necrotic lesions which occur on the leaves of certain varieties of the common garden bean, *Phaseolus vulgaris*, when they are inoculated with the virus of tobacco mosaic, and to suggest a possible use of these lesions in measuring virus concentration.

In securing virus for use as inoculum, care was taken to obtain samples that contained no virus other than that of tobacco mosaic. Samples of virus obtained from several different sources were used. All these samples resulted in the production of similar lesions on inoculated leaves of susceptible bean plants. The number of lesions produced on the leaves was roughly proportional to the concentration of virus used as inoculum. There was a multiplication of the virus in leaves on which local lesions developed. It is therefore believed that the disease described was caused by the virus of tobacco mosaic.

METHOD

The method of inoculation used in the experiments reported herein was similar to that used by Holmes in his work on *N. glutinosa*. A cheese-cloth pad, saturated with virus solution, was rubbed once over the entire upper surface of each bean leaf. The inoculated leaves were washed immediately with tap water in order to remove any excess virus.

Plants were grown in pots for eight to twelve days before inoculation. In general, the first young compound leaves were just appearing at this time.

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

Pea (navy) bean seed used in these experiments was obtained from a local grocery store. For this reason, the pea bean was treated separately and is not considered here as a distinct variety. Seed of the 77 varieties listed in table 1 was secured from four reliable seedsmen.

DEVELOPMENT OF LOCAL LESIONS ON THE PEA (NAVY) BEAN

The pea bean was used in the first attempt to inoculate leaves of bean plants with tobacco mosaic virus. Simple leaves of 14 pot-grown plants of the pea bean were inoculated soon after the first compound leaves made their appearance. The source of inoculum was a pure strain of the common field type of tobacco mosaic diluted 1 : 20 with water and kept frozen at -6° to -10° C. for four years. Two days after inoculation, 50 to 200 necrotic lesions appeared on each of the inoculated leaves. These lesions were one-half millimeter in diameter or smaller and each consisted of a pale necrotic area surrounded by a ring of dark red tissue. They increased slightly in size but never became much larger than one-half to one millimeter in diameter. Six similar plants inoculated in the same manner but with water as inoculum did not develop lesions. Sixteen other bean plants inoculated with juice from healthy tobacco plants remained free of lesions. Similarly, four uninoculated plants remained free of lesions. To confirm this result, 12 plants were inoculated with an undiluted virus extracted from diseased *N. tabacum* plants. All developed lesions two days later. Eight plants inoculated with water and four plants uninoculated did not develop lesions. These experiments indicate that tobacco mosaic can be transmitted to the pea (navy) bean. Experiments on the transmission of the virus of tobacco mosaic from beans to tobacco are discussed in another part of this paper.

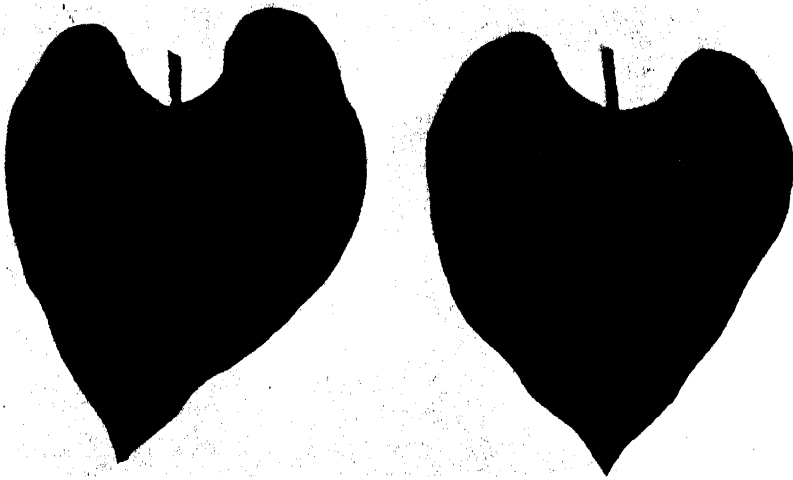
VARIETAL SUSCEPTIBILITY

Transmission of tobacco mosaic virus to pea beans immediately suggested the possibility of transmission to other varieties of beans. Consequently, 77 commercial varieties of *Phaseolus vulgaris* were tested for susceptibility and resistance. Plants for this test were grown in four-inch pots until the first compound leaves had fully developed. The simple leaves, which are the first to develop above the cotyledons, were inoculated with an undiluted virus secured from mosaic *N. tabacum* plants that had become infected three weeks earlier from inoculation with a 1 : 100,000 dilution of tobacco mosaic virus. The results of this test are presented in table 1, in which the number of plants of each variety inoculated, the number of plants on which lesions appeared, and the number of check plants are listed. These varieties are separated into three groups depending upon their susceptibility. In the first group, every plant inoculated developed local necrotic lesions; in the second group, lesions appeared on one or more plants of each variety inoculated; in the third group, none of the plants inoculated developed lesions and all were apparently healthy at the end of

six days. Plants in the third group were reinoculated on the sixth day in order to detect any extremely resistant variety not infected in the first attempt. Compound leaves were now inoculated with virus from the same source as that used in the first attempt. With one exception, all the plants reinoculated remained healthy for as long as 14 days. This exception was one of 16 reinoculated plants of the variety Kentucky Wonder which developed numerous necrotic lesions on the inoculated leaf. An explanation for this behavior has not been found but it is possible that seed of the variety was impure or not true to type.

Part of the above experiment was repeated using tobaccó mosaic virus from a different source and the 15 varieties found to be susceptible. Plants of each variety were inoculated with an undiluted virus which had been frozen at -6° to -10° C. for more than two weeks. The results of this experiment, presented in table 2, confirm those of the first test.

In addition, plants of *Vicia faba* and *Vigna sinensis* were tested at the



TEXT FIG. 1. Typical lesions produced on leaves of Scotia bean following inoculation with tobacco mosaic virus. The leaf at the right was not inoculated.

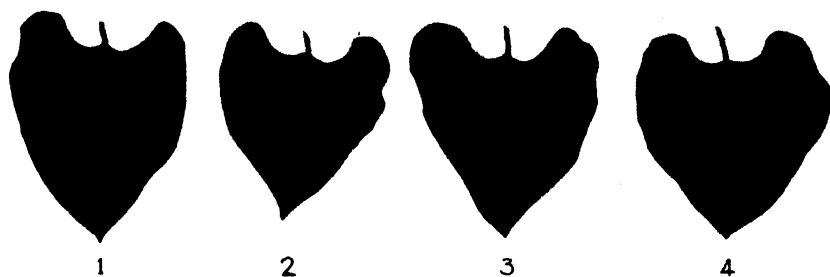
same time as plants listed in table 1. No lesions were developed on plants of either of these species.

Lesions on all susceptible varieties resembled in appearance those described on pea beans. The lesions varied slightly in size and considerably in number on the different varieties. In some varieties, only eight or ten lesions were produced on each inoculated leaf; in others, several hundred lesions developed on each leaf. Text figure 1 shows typical lesions produced on the variety Scotia.

Infected plants were under observation for at least three weeks during which time no indication of a systemic infection was noticed. Leaves formed after plants had been inoculated developed neither mottling nor necrotic lesions and appeared normal in every respect.

TRANSFER OF VIRUS FROM BEAN TO TOBACCO

An attempt was made to transfer tobacco mosaic virus from the lesions on bean leaves to tobacco plants. The inoculum used for this test was obtained from varieties which exhibited a large number of lesions on inoculated leaves. An infected leaf was folded in cheesecloth, pounded to express the juice, and rubbed over the upper surface of each leaf of one or more *N. tabacum* plants and one *N. glutinosa* plant. Check plants were treated in exactly the same way except that the inoculum used was obtained from inoculated leaves of varieties which did not develop necrotic lesions. The results of this experiment are shown in table 3. Since all the leaves used



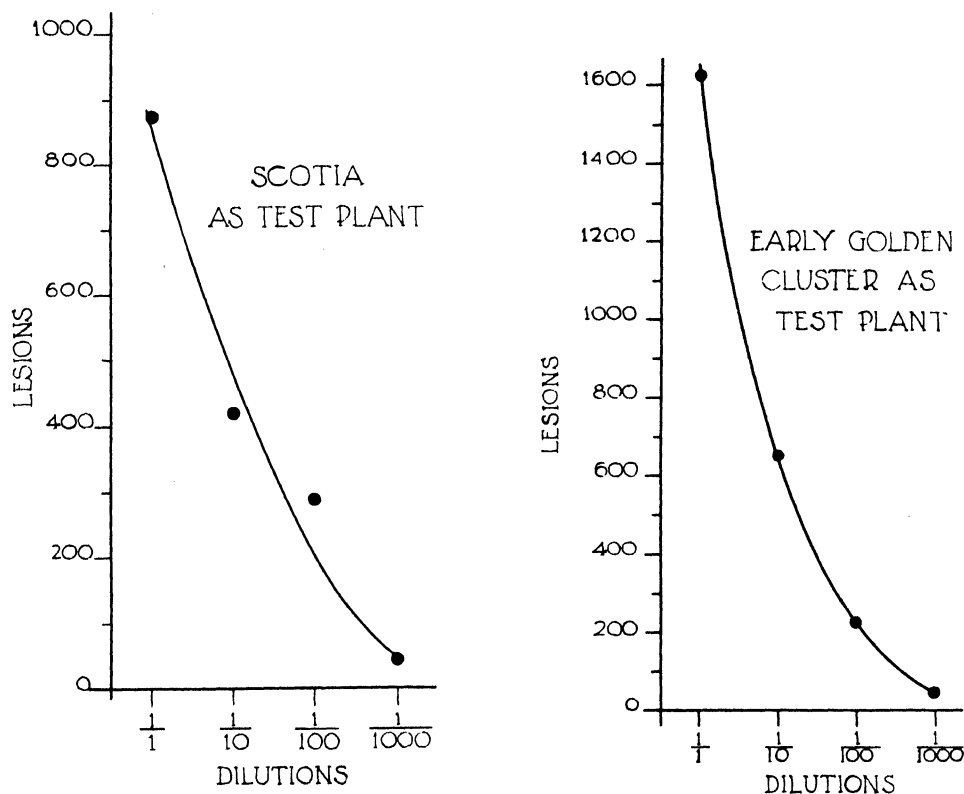
TEXT FIG. 2. Lesions produced on leaves of Early Golden Cluster beans following inoculation with tobacco mosaic virus. Leaf 1 was inoculated with an undiluted virus, leaf 2 with a 1 : 10 dilution, leaf 3 with a 1 : 100 dilution and leaf 4 with a 1 : 1000 dilution.

in this test had been previously inoculated with an undiluted virus it might be expected that all of them would retain a small amount of virus. However, considerably more virus would be expected in any leaves in which multiplication might have occurred. The relatively large number of lesions produced on leaves of *N. glutinosa* when inoculated with juice from leaves of Scotia and Early Golden Cluster is evidence that the virus had multiplied in leaves of these plants. The fact that all 11 plants inoculated with juice

from diseased leaves developed symptoms of tobacco mosaic, while only three of the 15 plants inoculated with juice from leaves on which no lesions occurred became infected, is significant and indicates that the virus had multiplied in the infected plants tested.

DILUTION EXPERIMENTS

If the number of lesions appearing on the leaves of inoculated plants can be correlated with the concentration of virus used as inoculum, it might be possible to use the number of lesions as a measure of virus concentration.



TEXT FIG. 3. *Phaseolus vulgaris* var. Early Golden Cluster as test plant. Figures 3 and 4 show the effect of diluting tobacco mosaic virus samples. In each case, the number shown represents the average number of lesions appearing for each leaf when eight leaves are rubbed with the virus sample tested.

TEXT FIG. 4. *Phaseolus vulgaris* var. Scotia as test plant.

In tests to determine whether such a correlation exists, plants of the varieties Scotia and Early Golden Cluster were used because the lesions appearing on the leaves of these varieties are numerous and large enough to be easily counted. Juice was extracted from mosaic tobacco plants, immediately diluted with water, and kept frozen until used for inoculation. After the first compound leaves had appeared, eight plants of each of the varieties

were inoculated by rubbing a given dilution of virus over the upper surfaces of the leaves. The dilutions of virus samples used in this test were, undiluted, 1 : 10, 1 : 100, and 1 : 1000. The result of the test is shown on leaves pictured in text figure 2. Lesions appeared on these plants the second day after inoculation but were not counted until the fifth day when they were somewhat larger. The number of lesions on each inoculated leaf is graphically shown in text figures 3 and 4. These figures show that the number of lesions decreases as the virus sample becomes more dilute. More extensive experiments are necessary to determine how well this

TABLE 1. *Susceptibility of Bean Varieties to Tobacco Mosaic **

| Variety Inoculated | No. of Plants Inoculated | No. of Plants Infected | No. of Plants Inoculated with Water | No. of Plants not Inoculated |
|-------------------------------------|--------------------------|------------------------|-------------------------------------|------------------------------|
| Group 1: | | | | |
| 1. Early Golden Cluster | 8 | All | 4 | 4 |
| 2. Ideal Market | 8 | " | 2 | 4 |
| 3. Scotia | 8 | " | 4 | 4 |
| 4. Cut Short or Corn Hill | 8 | " | 4 | 4 |
| 5. White Creaseback | 6 | " | 4 | 4 |
| 6. Stringless Refugee | 7 | " | — | 2 |
| 7. Hodson Long Pod | 5 | " | 1 | 4 |
| 8. Keeney's Stringless Refugee | 7 | " | — | 4 |
| 9. Refugee Green Pod | 7 | " | 4 | 3 |
| 10. New Navy Robust | 1 | " | — | — |
| Group 2: | | | | |
| 11. Unrivalled | 8 | 7 | — | 1 |
| 12. Improved Round Pod Valentine | 8 | 1 | 4 | 4 |
| 13. Great Northern | 8 | 4 | 2 | 4 |
| 14. Refugee Extra Early | 7 | 4 | — | 5 |
| 15. Full Measure | 6 | 1 | 3 | — |
| Group 3: | | | | |
| 16. Bountiful | 7 | 0 | 3 | — |
| 17. Dwarf Horticultural | 7 | 0 | 4 | 3 |
| 18. Early Red Valentine | 8 | 0 | 2 | — |
| 19. King of the Earlies | 6 | 0 | 2 | 2 |
| 20. Longfellow | 7 | 0 | 4 | 4 |
| 21. Masterpiece | 8 | 0 | — | 4 |
| 22. Tendergreen | 7 | 0 | 4 | 4 |
| 23. Henderson Stringless | 1 | 0 | 4 | — |
| 24. White Marrow | 7 | 0 | 3 | — |
| 25. White Kidney | 3 | 0 | 3 | — |
| 26. Red Kidney | 8 | 0 | 4 | — |
| 27. Giant Stringless | 8 | 0 | 4 | 3 |
| 28. Tennessee Green Pod | 3 | 0 | — | 1 |
| 29. Sutton's Masterpiece | 7 | 0 | 3 | 4 |
| 30. Low's Champion or Red Cranberry | 7 | 0 | 3 | 2 |
| 31. Improved Black Wax | 8 | 0 | 4 | 3 |
| 32. Golden Age | 7 | 0 | 1 | 1 |
| 33. Sure Crop Stringless | 4 | 0 | 3 | — |
| 34. Wardell's Kidney | 4 | 0 | 3 | 1 |
| 35. Burpee's New Kidney | 1 | 0 | 1 | — |
| 36. Monster Stringless | 3 | 0 | 2 | — |
| 37. Round Pod Kidney | 12 | 0 | — | 1 |
| 38. Prolific German Black Wax | 4 | 0 | 1 | — |
| 39. Violet Wax | 1 | 0 | — | 1 |

TABLE I.—*Continued*

| Variety Inoculated | No. of Plants In- oculated | No. of Plants Infected | No. of Plants In- oculated with Water | No. of Plants not Inoculated |
|--|----------------------------------|------------------------------|---|------------------------------------|
| 40. New White Stringless..... | 4 | 0 | — | — |
| 41. Improved Golden..... | 3 | 0 | 4 | — |
| 42. Pencil Pod..... | 7 | 0 | 4 | 4 |
| 43. Davis Kidney..... | 4 | 0 | — | — |
| 44. Webber Wax..... | 4 | 0 | 1 | — |
| 45. Currie's Golden..... | 7 | 0 | 3 | 4 |
| 46. Currie's Rust Proof Black Wax..... | 6 | 0 | 2 | — |
| 47. Davis White Wax..... | 7 | 0 | 2 | 1 |
| 48. Dwarf Golden Carmine..... | 4 | 0 | 3 | 1 |
| 49. Improved Rust Proof Golden..... | 4 | 0 | 2 | 1 |
| 50. Horticultural..... | 8 | 0 | 4 | — |
| 51. Horticultural Cranberry..... | 8 | 0 | 4 | 4 |
| 52. Lazy Wife..... | 4 | 0 | 2 | — |
| 53. Scarlet Runner..... | 8 | 0 | 4 | 4 |
| 54. Burger's Stringless..... | 5 | 0 | 2 | 2 |
| 55. White Dutch Runner..... | 4 | 0 | 2 | — |
| 56. Kentucky Wonder..... | 16 | 0 | 6 | 5 |
| 57. Fordhook..... | 4 | 0 | 1 | — |
| 58. Henderson's Bush Lima..... | 5 | 0 | 3 | — |
| 59. Henderson's Early Giant..... | 4 | 0 | 1 | — |
| 60. New Wonder..... | 2 | 0 | 1 | — |
| 61. Burpee's Improved..... | 2 | 0 | 1 | — |
| 62. Early Leviathan..... | 3 | 0 | 2 | — |
| 63. Large White Lima..... | 3 | 0 | 1 | — |
| 64. Henderson's New Ideal..... | 1 | 0 | — | — |
| 65. Large Lima..... | 1 | 0 | — | — |
| 66. King of the Garden..... | 4 | 0 | 3 | — |
| 67. Extra Early Jersey..... | 4 | 0 | 2 | — |
| 68. Ford's Mammoth..... | 3 | 0 | 1 | — |
| 69. Carpinteria..... | 7 | 0 | 2 | — |
| 70. Large Green Seeded Lima..... | 2 | 0 | 3 | — |
| 71. Siebert's Early..... | 6 | 0 | 1 | 2 |
| 72. Ideal Lima..... | 3 | 0 | 1 | — |
| 73. Dreer's Improved Pole..... | 5 | 0 | 1 | 1 |
| 74. Early Jersey..... | 1 | 0 | 1 | — |
| 75. Sieva..... | 3 | 0 | 2 | — |
| 76. Dreer's Bush..... | 5 | 0 | 2 | — |
| 77. Dreer's Wonder Bush..... | 7 | 0 | 1 | — |

* This table shows the bean varieties on which local necrotic lesions are produced when the leaves are rubbed with virus of tobacco mosaic. All check plants shown in the last two columns remained free of lesions.

correlation holds under various conditions. The tests indicate that it will be possible to work out a standard measure of virus concentration using plants of one or more of the susceptible bean varieties.

DISCUSSION

The fact that bean plants are easily and quickly grown from seed makes them desirable for use in measuring tobacco mosaic virus concentration. Two or three months are required to grow *N. glutinosa* plants to the stage at which they are suitable for inoculation. Bean plants, however, require

only eight or ten days to reach this stage. Obviously, the saving in time is considerable.

Many varieties of beans have proven to be susceptible to the virus of bean mosaic. Plants affected with this mosaic have a distinct mottled appearance quite different from the appearance of plants on which necrotic

TABLE 2. *Varietal Susceptibility **

| Variety Inoculated | No. of Plants Inoculated | No. of Plants Infected | No. of Plants Inoculated with Water | No. of Plants Inoculated with Healthy Juice |
|---------------------------------------|--------------------------|------------------------|-------------------------------------|---|
| 1. Early Golden Cluster..... | 11 | All | 4 | 4 |
| 2. Ideal Market..... | 12 | " | 4 | 4 |
| 3. Scotia..... | 12 | " | 4 | 4 |
| 4. Cut Short or Corn Hill..... | 12 | " | 4 | 3 |
| 5. White Creaseback..... | 8 | " | 3 | 2 |
| 6. Stringless Refugee..... | 9 | " | 3 | 4 |
| 7. Hodson Long Pod..... | 12 | " | 3 | 3 |
| 8. Keeney's Stringless Refugee..... | 7 | " | 3 | 3 |
| 9. Refugee Green Pod..... | 7 | " | 4 | 3 |
| 10. New Navy Robust..... | 7 | " | 1 | 4 |
| 11. Unrivalled..... | 8 | None | 3 | 3 |
| 12. Improved Round Pod Valentine..... | 9 | 1 | 4 | 4 |
| 13. Great Northern..... | 11 | All | 4 | 1 |
| 14. Refugee Extra Early..... | 8 | 2 | 4 | 4 |
| 15. Full Measure..... | 10 | 3 | 4 | 4 |

* The table confirms the results of groups 1 and 2 of table 1. Check plants inoculated with water (column 3) and plants inoculated with juice from healthy tobacco (column 4) remained free from lesions.

TABLE 3. *Transfer of Tobacco Mosaic Virus from Lesions on Bean Leaves Back to Tobacco Plants **

| Variety Used as a Source of Inoculum | No. of <i>N. tabacum</i> Plants Inoculated | No. of <i>N. tabacum</i> Plants Infected | No. Lesions on <i>N. glutinosa</i> |
|--------------------------------------|--|--|------------------------------------|
| Many lesions following inoculation: | | | |
| 1. Ideal Market..... | 1 | 1 | 1 |
| 2. Extra Early Refugee..... | 2 | 2 | 6 |
| 3. Great Northern..... | 1 | 1 | 7 |
| 4. Scotia..... | 1 | 1 | 49 |
| 5. Early Golden Cluster..... | 1 | 1 | 35 |
| No lesions following inoculation: | | | |
| 6. White Dutch Runner..... | 2 | 0 | 0 |
| 7. Horticultural Cranberry..... | 2 | 2 | 0 |
| 8. Prolific German Black Wax..... | 2 | 1 | 0 |
| 9. Monster Stringless..... | 2 | 0 | 0 |
| 10. Longfellow..... | 2 | 0 | 0 |

* The table shows the number of *N. tabacum* plants infected and the number of lesions developed on single plants of *N. glutinosa* when their leaves are rubbed with juice from inoculated bean leaves. Varieties 1-5 are those on the leaves of which lesions were produced following inoculation with mosaic virus. Varieties 6-10 are those on the leaves of which no lesions occurred following inoculation.

lesions are produced as a result of inoculation with tobacco mosaic virus. Reddick and Stewart (2, 3) tested a large number of varieties for resistance to bean mosaic. It is interesting to note that there is no correlation between susceptibility to tobacco mosaic and susceptibility to bean mosaic. Some varieties, such as White Creaseback, susceptible to tobacco mosaic, are immune to bean mosaic. Other varieties, such as Red Kidney, which are immune to tobacco mosaic, are susceptible to bean mosaic. Still other varieties, such as White Marrow, are resistant to both.

Local lesions caused by the virus of the ring spot disease of tobacco have been reported by Wingard (4) on kidney bean and on lima bean. Neither of these developed lesions when plants were inoculated with virus of tobacco mosaic. Plants of some of the varieties of beans susceptible to infection with tobacco mosaic virus have been inoculated with a sample of ring spot virus. The lesions produced by this virus are similar to but distinctly different from those caused by tobacco mosaic on the same varieties.

SUMMARY

1. Certain varieties of the common garden bean, *Phaseolus vulgaris*, develop local necrotic lesions when juice containing virus of the ordinary field type of tobacco mosaic is rubbed onto the upper surfaces of the leaves.

2. More virus was recovered from bean leaves on which lesions occurred than from similarly inoculated leaves of immune varieties on which no lesions occurred. This indicates that multiplication of virus took place in leaves of susceptible varieties.

3. Fewer lesions appear on leaves inoculated with dilute samples of virus than on leaves inoculated with concentrated samples. Curves are presented which indicate the possibility of using the number of lesions on susceptible varieties of beans as a measure of virus concentration.

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THE MORPHOLOGY AND ANATOMY OF THE ACHENE

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The majority of botanists have long since agreed that the achene has been derived from the follicle by reduction. However, supporters of the opposing view, namely, that the multiovular structure (follicle) has been derived from the uniovular (achene) are not lacking (4). The present investigation has been undertaken to look for proof of this supposed reduction and to determine the morphological and anatomical changes which have occurred. Both the morphological and the anatomical evidence support the view that the achene is a reduced follicle. The morphological changes are a marked decrease in the size of the carpel and in the number of ovules per carpel, the multiovular carpel becoming uniovular. The anatomical changes involve the reduction of the vascular tissue both in the number of traces which a carpel receives and in the traces themselves within the carpel.

The Ranunculaceae and the Rosaceae were chosen as most suited to this study, since they provide genera with a considerable variety of follicle and achene types.

A follicle is "the fruit of a single carpel dehiscent by one (the ventral) suture." It contains from few to many seeds attached along its two margins. The typical carpel (or follicle), being a modified leaf, receives the vascular supply of a typical angiosperm leaf, namely three bundles: the median of these is the dorsal trace and plays no part in the ovular supply; the other two, variously known as "marginal" or "ventral traces" (6), give off a branch to each ovule attached to the margins along which they run. Branches from all three bundles may or may not be given off to the ovary wall. The follicle has, therefore, an abundant supply of vascular tissue.

An achene is a "small, dry, indehiscent, one-seeded, seed-like fruit or carpel in which the covering does not adhere to the seed, as in the sunflower or buttercup." Unlike the follicle, the achene does not receive a typical supply of vascular tissue. It has usually only a single trace.

The many-seeded condition, such as is found in the follicle, is commonly

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regarded as primitive, and the one-seeded condition of the achene as derived from that of the follicle by reduction. In this reduction there seems to have been a more or less gradual decrease in the number of ovules. There are many-seeded carpels among the most primitive members of the two families under consideration, such as *Aquilegia*, *Caltha*, *Trollius*, *Spiraea*, *Physocarpus*; and typical one-seeded forms are found in *Ranunculus*, *Anemone*, *Clematis*, *Waldsteinia*, *Potentilla*, *Fragaria*.

Evidences of reduction are not wanting. Rudimentary ovules occur regularly in *Clematis*, *Hepatica*, and *Anemone* (3, 4, 10). A strong, well developed ovule accompanied by a weaker one, with only one ripening into a seed, is regularly found in *Rubus* and *Dalibarda*, frequently in *Hydrastis*. An ovular trace departing to a suppressed ovule is noted by the writer in *Waldsteinia* and *Dalibarda*. Smith (17) reports the finding of evidence of one or more suppressed ovules at the top of the ovary in *Trollius*, as is shown by the presence of ovular traces.

If this reduction from a follicle to an achene has taken place, that is, if the multiovular condition has become uniovular, what has become of the extensive vascular supply of the former? How is the single-traced condition of the achene to be accounted for? And how is the fact that the ovular trace in some achenes is apparently derived from the dorsal bundle rather than from a ventral to be accounted for?

As before stated, three traces or bundles constitute a typical angiosperm leaf supply; generally these leave three gaps in the stele. Sinnott (16), as a result of his investigations on the anatomy of the node in angiosperms, concludes that for leaves the three-trace condition is primitive and that a change from this to the one-trace condition is due to reduction. He further states that this reduction may occur in two ways: either by a dropping out of the two lateral traces, thus leaving the central trace as the one supplying the leaf; or by a fusion of the two laterals with the central, thus furnishing the leaf with a single trace, fundamentally of triple nature.

The investigations carried on by the writer lead to the conclusion that the one-trace supply of the achene of Ranunculaceae and of Rosaceae, like that of the one-trace leaf, is really a fusion bundle composed of a median (dorsal) and two lateral (ventral) traces. Evidence to support this statement is presented in the following pages.

SPECIES STUDIED

In the present investigation there were studied the following species in which the fruit is a follicle:

Aquilegia canadensis L.

Cimicifuga racemosa (L.) Nutt.

Trollius laxus Salisb.

Helleborus viridis L.

Delphinium cultorum Voss.

Coptis trifolia (L.) Salisb.
Caltha palustris L.
Hydrastis canadensis L.

The following species were studied in which the fruit is an achene:

Ranunculaceae

Ranunculus Ficaria L.
R. longirostris Godr. (*R. circinatus* Sibth.)
R. aquatilis L. var. *capillaceus* DC.
R. Cymbalaria Pursh.
R. delphinifolius Torr.
R. sceleratus L.
R. abortivus L.
R. recurvatus Poir.
R. fascicularis Muhl.
R. septentrionalis Poir.
R. hispidus Michx.
R. repens L.
R. pennsylvanicus L. f.
R. acris L.
R. bulbosus L.
R. reptans L., var. *ovalis* (Bigel.) T. and G. (*R. Flammula* L., var. *reptans* (L.) May.)
R. Flammula L., var. *unalaschensis* (Bess.) Ledeb.
R. Bongardi Greene (*R. tenellus* Nutt.)
Myosurus minimus L.
Thalictrum dioicum L.
T. polygamum Muhl.
T. dasycarpum Fisch. and Lall.
T. occidentale Gray.
T. revolutum DC.
Anemonella thalictroides (L.) Spach.
Anemone quinquefolia L.
A. canadensis L.
A. virginiana L.
Hepatica acutiloba DC.
H. americana (DC.) Ker. (*H. triloba* Chaix.)
Clematis verticillaris DC.
C. virginiana L.

Rosaceae

Fragaria vesca L.
F. virginiana Duch.
Duchesnea indica (Andr.) Focke.

Waldsteinia fragarioides (Michx.) Tratt.
Potentilla canadensis L.
P. recta L.
P. norvegica L., var. *hirsuta* (Michx.) Lehm. (*P. monspeliensis* L.)
Geum canadense Jacq.
G. strictum Ait.
G. rivale L.
Rubus pubescens Raf. (*R. triflorus* Richards)
R. idaeus L., var. *strigosus* (Michx.) Maxim. (*R. idaeus*, var. *aculeatissimus* [C. A. Mey] Regel and Tiling.)
R. hispidus L.
R. flagellaris Willd. (*R. villosus* Ait.)
R. allegheniensis Porter.
Dalibarda repens L.
Agrimonia striata Michx.

The Ranunculaceae as members of the Ranalian plexus are of particular interest because of their probable phylogenetic position. Evidence tends to point toward these forms as being among the most primitive of existing angiosperms. The follicle of this family is therefore very probably a primitive type of carpel. Its vascular supply should be similar to that of the primitive carpel since among the most conservative structures of a plant seem to be its vascular system and reproductive parts.

The present investigation concerns the vascular supply of the achene which is looked upon as a reduced follicle. Therefore, before studying the achene, the vascular supply and the ovule situation of the follicle in the Ranunculaceae will be given in detail. Besides the species already listed reference will occasionally be made to *Isopyrum*, *Xanthorrhiza*, and *Calli-anthemum*. The writer regrets that first-hand studies could not be made of species of these genera.

In this investigation the term "trace" is used in a strictly anatomical sense. It is applied only to those bundles which leave the stele of the receptacle, traverse the cortex, and enter the base of the carpel; and to the bundle which, departing from a ventral strand, enters an ovule. The terms "strand" or "bundle" are used indiscriminately to indicate any vascular tissue within the carpel.

SPECIES WITH FRUIT A FOLLICLE

Aquilegia canadensis

This species has been chosen as typical of the vascular supply and of the ovule situation in the follicle of Ranunculaceae. There are minor variations between *Aquilegia* and the other genera studied in the manner in which the traces depart from the receptacle, the length of the strands within the carpel, and the abundance of vascular tissue in the ovary wall, but none in the way in which the ovules receive their traces.

The five gaps which mark the "passing out" of the five dorsals from the stele appear very soon after the departure of the last stamen traces (Pl. XLII, fig. 1). Five masses of vascular tissue are left; each of these divides, the half on either side of a dorsal (fig. 2) proceeding into the carpel to which that dorsal has already departed, thus giving it a three-trace supply, a dorsal and two ventrals (fig. 3). All of the vascular tissue within the receptacle is used up with the departure of the last trace to a carpel. In due course each of the many ovules receives a trace from the strand which supplies the margin to which the ovule is attached. These traces tend to go off alternately, but they do not do so necessarily. As a general thing, the ovary wall is well supplied with branches from the three principal strands. These three strands continue up the style to the base of the stigma.

Coptis trifolia and *Caltha palustris* resemble *Aquilegia canadensis* in all essentials.

Delphinium cultorum

This species differs from the type chosen in the manner in which the carpel supply is given off. About the level at which the carpels begin to form, the stelar bundles become arranged in three groups of three strands each (fig. 4). These pass directly into the base of the ovary. There is no long passage of the traces through the cortex as in all other species examined except *Cimicifuga*. Each of these groups of vascular tissue forms the three-trace supply of a single carpel.

Hydrastis canadensis

Hydrastis differs from all the other follicular plants studied in that it is biovular instead of multiovular; each carpel margin bears one ovule. As a general thing one ovule is more strongly developed than the other. The vascular system is like that of *Aquilegia canadensis*.

Helleborus viridis

In general the carpel situation in this species closely approximates that of the type chosen. An interesting, unusual condition, however is found in several carpels. These unusual carpels possess the normal three traces at the stele of the receptacle (fig. 5y), but the two ventral traces soon fuse and enter the base of the carpel as a single strand (fig. 6y); as a single strand they proceed up the margin a varying distance before their double nature is again apparent (fig. 7y). After the split the two strands thus formed follow the normal course of ventral strands in follicles. In the lateral fusion of the two ventral traces for part of their course this plant shows apparently the first step in the fusion of the three traces of the carpel supply.

Cimicifuga racemosa

Certain unusual features were found in the trace supply of this species, but the author is deferring discussion of these until a later paper.

Ovule Reduction

In *Trollius* Smith (17) finds that "above the ovules strands pass inward from the ventral traces to the ovular cavity and end abruptly." Concerning this he says, "The lower of these [strands] enter ovules, but the upper ones end abruptly at the margin of the cavity. Thus the form, course, and position of these traces suggest that they are supplies to suppressed ovules."

The general situation in follicles strongly suggests that a reduction in the number of ovules has occurred. There are the multiovular forms, *Helleborus*, *Cimicifuga*, and *Coptis* (Pl. XLIV, fig. 53), with an ovary so crowded full of ovules that there is no suggestion of suppression; closely allied to these are *Aquilegia*, *Caltha*, *Trollius*, and *Delphinium* (fig. 54), with a wide empty space above the last ovule suggesting the possibility that once these ovaries were filled. Smith's (17) finding in *Trollius* bears out the above statement.

From the multiovular condition of the above there is a drop to the biovular condition of *Hydrastis* (fig. 55), *Xanthorrhiza*, and *Callianthemum*.

Isopyrum (fig. 56) would seem to be a connecting link between the multi- and the uniovular conditions, for here Baillon (3) reports some carpels with several ovules, some with only one. Each margin of the follicle of *Hydrastis*, *Xanthorrhiza*, and *Callianthemum* bears a single ovule; in *Hydrastis* one ovule is noticeably weaker than the other; in *Xanthorrhiza* and *Callianthemum* one regularly aborts. The uniovulate carpel of *Isopyrum* would then mark the extreme reduction in the number of ovules in the follicle.

The Vascular Supply of the Follicle

The normal carpel receives a three-trace vascular supply (fig. 53). Each trace enters the base of the carpel as a single strand; and as a single strand each proceeds up the ovary wall and on into the style. There is no fusion except as some of the branches anastomose.

Several carpels were found in *Helleborus* in which the ventrals are fused almost from the beginning. In such carpels (fig. 57) only two bundles, therefore, enter the ovary, the dorsal and the fused ventrals. These ventrals remain fused for a varying distance up the ovary wall. This is clearly a case of reduction of the carpel supply, only two bundles entering the carpel instead of the usual three. Thus fusion of the carpel traces in reduction seems to begin by a lateral fusion above the gaps.

Smith (17) reports a case of similar but more extreme reduction in *Aquilegia canadensis*. The follicle described, contrary to the usual condition found in follicles, receives only a single trace (fig. 58). This single trace, as he shows, is a fusion trace which has been formed by the lateral fusion of all three traces, the two ventrals and the dorsal.

Conclusions

The morphological studies of the follicles of Ranunculaceae lead to the conclusion that the multiovular condition, through reduction and sup-

pression, leads to the uniovular. The anatomical study supports this conclusion and shows that in the reduction the traces of the carpel tend to fuse, the two ventrals forming one trace, or the two ventrals and the dorsal fusing to form a single trace. The carpel may then have at its base two traces or only one.

SPECIES WITH FRUIT AN ACHENE

Waldsteinia fragarioides

The vascular supply of the achene of *Waldsteinia* (fig. 74) bears a very close resemblance to that of a follicle. Just as in a follicle, three traces leave the stele of the receptacle and enter the carpellary base. These traces become the dorsal and ventral strands of the carpel. They move up the ovary wall in the usual manner to the base of the stigma; each ventral gives rise to an ovule trace. The first trace is to a suppressed ovule, the second passes into the functional ovule. Both ovule and ovary are very abundantly supplied with vascular tissue, branches from the main strands. More or less anastomosis occurs among these branching strands.

Ranunculus acris

Plate XLIII, figure 29, is a diagrammatic representation of a longitudinal section of an achene showing the ovule trace (*d*), the carpel trace (*c*), the dorsal bundle (*b*), and a ventral bundle (*a*), all in the plane of section; a branch (*e*) from the dorsal is out of the plane of section. Within the carpel base the carpellary trace (*c*) divides at once into two main parts one of which (*b*) "dips" quickly to the dorsal side, at the same time gives off two lateral branches (*e*) which move more or less parallel with it up the ovary wall. The median bundle (*b*), which is the dorsal strand, continues up the style. Its branches (*e*), for the purpose of identification, will be called "dorsal-lateral strands." At the same level the remaining branch of the original trace divides, and the two strands (*a*) thus formed proceed upward, side by side, along the ventral margin. In due course one of these gives off a branch (*d*) which passes directly into the ovule (fig. 37). This branch is the ovule trace. The strand from which it comes is a ventral strand. The other branch, likewise a ventral, gives rise to no ovule trace. Above the ovule cavity the ventral and the dorsal-lateral on the same side fuse (figs. 29, 39, 40). The achene, then, is furnished with a five-strand vascular system, all five strands being equally prominent.

Ranunculus Ficaria

This species (fig. 42) differs from *R. acris* (fig. 29) in two respects: (1) the ovule trace (*d*) leaves the ventral (*a*) at a lower level, that is, it comes off closer to the point of union of the fused ventrals and the dorsal; (2) the dorsal-laterals are not present. This achene, then, is supplied with a three-strand vascular system, as opposed to *R. acris* with its five strands. As in

R. acris, these three strands are equally strong. Some carpels show a significant situation in the departure of the trace from the stele. The carpal trace supply arises not as usual as a single trace, but in three parts (fig. 41), two strong lateral ones and a weaker median one. These traces fuse in the cortex very soon after leaving the stele, so that they enter the base of the carpel as a single trace. This clearly suggests that the single trace is fundamentally composed of three.

Ranunculus repens

Figure 43 is a diagrammatic longitudinal section drawn as in *R. acris*. The one trace (*c*) which enters the base of the carpel divides immediately. One of these divisions which is the dorsal strand (*b*), bears to the dorsal side and branches, as already described in *R. acris*. It continues to the base of the stigma. The other branch, which represents the fused ventral strands, forks at once into three strands, a median and two laterals. The median, which is the ovule trace (*d*), proceeds directly into the ovule. The remaining two are the ventrals (*a*). As such they move up the ventral edge of the ovary wall. Above the ovular cavity they anastomose with the dorsal-laterals. In *R. acris* the ventrals do not extend beyond this fusion; in *R. repens* they continue with the dorsal up the style. It is worthy of note that in *R. repens* the ovule trace leaves the ventral at a still lower level than it did in *R. Ficaria*. It comes off very close to the point of union of the fused ventrals and the dorsal. In all of the following species the ovule trace departs from this point.

Ranunculus septentrionalis and *Ranunculus bulbosus*

The main vascular system of these two species is exactly like that of *R. repens*. Minor variations occur in the form of anastomosing short branches, which end blindly in the ovary wall.

Ranunculus pennsylvanicus

R. pennsylvanicus (fig. 44) differs from *R. repens* (fig. 43) mainly in that the ventrals (*a*) do not extend into the style; they end at the anastomosis with the dorsal-laterals (*e*) when the latter are present, otherwise they end above the ovular cavity at the base of the style. This species is of particular interest because of the two types of carpels found here: in one, the dorsal-laterals are present; in the other, the dorsal-laterals do not appear. This condition is strongly suggestive that this species is a transitional one in the reduction of the amount of vascular tissue present in an achene. One type has a five-strand system, the other a three-strand.

Ranunculus hispidus and *Ranunculus fascicularis*

The main vascular system of the achenes of these two species is like that of the five-strand achene of *R. pennsylvanicus*. They differ in having an ovary wall more abundantly supplied with branches from the dorsal and the ventral strands.

Ranunculus recurvatus

The achene of this species (Pl. XLIV, fig. 63) is like the three-strand achene of *R. pennsylvanicus* (fig. 64); it differs in having a dorsal strand which does not continue into the style but ends at its base.

R. delphinifolius and *R. Bongardi* differ in no essential points from *R. recurvatus*. *R. abortivus* differs from *R. recurvatus* in that it has a still shorter dorsal strand.

Myosurus minimus

The vascular system at the base of the achene of this species (fig. 69) bears a close resemblance to that of the three-strand achene of *R. pennsylvanicus* (fig. 64), that is, it is supplied with a dorsal strand, two ventral ones, and an ovular trace, all departing from the carpel trace at approximately the same level. The dorsal-laterals have lost their basal portions, but not their upper. They persist for a short distance beyond the point of fusion with the ventrals.

Ranunculus reptans var. *ovalis*

In this species (fig. 45) as in *R. recurvatus*, *R. delphinifolius*, *R. Bongardi*, *R. abortivus*, and all of the *Ranunculus* species which follow, there has been a complete dropping out of the dorsal-laterals; otherwise the bundle situation at the base of the carpel is as described in *R. repens*. The ventral strands (a) follow a course rather unusual in ventrals. After curving over the ovular cavity and proceeding down the opposite side they fuse with the dorsal (b). Henslow (12) notes a similar situation in *R. flammula*.

R. sceleratus is closely similar to *R. reptans*.

Ranunculus Flammula var. *unalaschensis*

This species (fig. 47) may be looked upon as a form somewhat transitional in the varying length of its ventrals (a). The ventrals range in extent from about one half the distance up the ventral wall to the top of the ovary cavity. The situation at the base of the achene is that of the three-strand achene of *R. pennsylvanicus* (fig. 44).

Duchesnea indica

This rosaceous species (fig. 76) presents essentially the same vascular situation as is found in a three-strand achene of *Ranunculus* where the ovule trace leaves the ventral at the point of fusion of the ventral strands with the dorsal. Its achene supply differs from that of *R. Flammula* var. *unalaschensis* (fig. 47), for example, only in the length of its dorsal, which is short, extending about one-half way up the dorsal wall; like *R. Flammula*, it has short ventrals.

Geum rivale (fig. 75), *G. strictum*, and *G. canadensis* bear a very close resemblance to each other and to the three-strand achene of *Ranunculus* as given in the preceding paragraph. In these rosaceous species the dorsal and the ventral strands extend up the style to the base of the stigma.

Geum is regularly reported as being biovulate, but the writer could find no suggestion of this condition either in the presence of rudimentary ovules or of an ovule trace to a suppressed ovule.

Ranunculus Cymbalaria (fig. 48) shows a marked decrease in the length of its ventrals (*a*); they are reduced to mere stubs.

In *R. longirostris* (fig. 46) reduction in the length of the ventral strands is complete. They have disappeared as such in that they never separate from the dorsal (*b*). Hence it is that the ovular trace (*d*) appears to come from the dorsal strand. This species also has a short dorsal strand. It ends about one-half way up the dorsal side of the ovary wall.

R. aquatilis is exactly like *R. longirostris* except that the dorsal continues into the style.

Thalictrum polygamum

This species (Pl. XLII, fig. 8) will be used as the type for *T. dioicum*, *T. dasycarpum*, *T. occidentale*, *T. revolutum*, and *Anemonella thalictroides*. All these plants have carpels with a thick ovary wall bearing eight to ten ridges, each ridge with its vascular supply which arises as a branch from either the dorsal or the ventral strand. The single trace (*c*) that enters the base of the carpel immediately forks; one bundle, the dorsal (*b*), proceeds up the carpel unbranched. The remaining bundle, the fused ventrals (*a*), gives off strands alternately to each of the ridges. All of these strands, usually eight, continue up the ovary wall (fig. 10). Well up towards the top of the ovular cavity, near the level at which the ovular trace departs, the two branches on either side of the ventral begin to move in towards the ventral (fig. 11). At this level the ventral splits into three parts (fig. 12), a median and two laterals. The median is the ovule supply; it curves over and downwards into the pendulous ovule (figs. 8*d*, 13). The laterals are the true ventrals, which up to this level have appeared only as one trace. These soon anastomose (fig. 14), each with the trace nearest it; that is, the ones which have moved in from the nearest ridges. Seven strands (fig. 14), instead of eight, now continue into the style; gradually these drop out until at the tip (fig. 15) only three are left, presumably the dorsal and the two ventrals.

It is not uncommon to find in this species a carpel which reveals the three-fold character of its trace (fig. 9). Three strands come off very close together; they soon fuse and thus enter the carpel base as a single trace. Within the base this trace splits, forming two equally strong bundles, a dorsal and a fused ventral.

T. dioicum, *T. occidentale*, and *T. revolutum* show the same anatomical situation within the carpel as described in *T. polygamum*.

T. dasycarpum and *Anemonella thalictroides* differ from *Thalictrum polygamum* in that the dorsal gives rise to several of the strands going to the ridges. *Anemonella thalictroides* differs still further in that the fused ventrals fork into two strands (fig. 17) instead of three. As the true ventrals these two strands continue up the ovary wall. Soon one of these gives off a

bundle (fig. 19), the ovular trace, which curving over the top of the cavity passes into the ovule (fig. 20). Above this level *A. thalictroides* is anatomically like *Thalictrum polygamum*. The multibundle condition of *Thalictrum* and *Anemonella* is due to the free branching of the ventral (generally) and the dorsal (occasionally) in the base of the carpel. This branching continues until all the ridges, eight to ten in number, are supplied.

Anemone canadensis

A. canadensis will serve as a type for *A. quinquefolia*, *A. virginiana*, *Clematis verticillaris*, *C. virginiana*, *Hepatica acutiloba*, and *H. americana*, all of which are very similar in achene vascular supply. The achenes of these plants, like those of *Ranunculus*, receive a single trace (fig. 22) which forks in the base of the carpel: one of these branches is the dorsal (*b*), the other the fused ventrals (*a*). As in *T. polygamum* these fused ventrals pass up the ovary wall as a single strand until near the top of the ovule cavity. Here the bundle divides into three strands. The median is the ovular trace (*d*); it curves over and downward into the pendulous ovule. The laterals are the free ventrals and as such continue up the style with the dorsal.

Clematis virginiana, *Anemone virginiana*, and an occasional carpel in *Hepatica acutiloba* differ from the above in having the ventrals end abruptly at the point where the ovular trace departs.

Just as in *Ranunculus Ficaria* (fig. 41) and *Thalictrum polygamum* (fig. 9), several carpels of *Anemone canadensis* show the composite nature of the carpel trace.

The carpels of *Anemone*, *Clematis*, and *Hepatica* are found to possess more than one ovule. Only one of these ovules, however, is functional; the others, varying in number from one to four, are always abortive (fig. 22x). The sterile ovules vary in size from minute appendages consisting of only one cell with several nuclei to well formed ovules with a full complement of cells. No sign of vascular tissue is found associated with these abortive ovules.

Potentilla recta

There is much resemblance anatomically between the achene of this species and that of *Anemone canadensis*. There is this difference, however. In *Anemone canadensis* the ventrals remain fused up to the level at which the ovule trace departs, whereas in *Potentilla recta* the ventrals separate at a lower level than that of the origin of the ovule trace. That is, if reduction involves fusion, then reduction has proceeded further in this particular ranunculaceous species than it has in the rosaceous one. Several carpels were found with rudimentary ovules. There is no indication of vascular tissue supplying these. The presence of these ovules is of interest because *Potentilla* is commonly described as having but one ovule.

Potentilla canadensis

This species is like *P. recta* except for the dropping out of the ventrals at the time of the departure of the ovule trace; that is, they do not extend into the style. Also, there are no rudimentary ovules.

P. norvegica var. *hirsuta* resembles *P. recta* except for the absence of a rudimentary ovule.

Rubus hispidus

The anatomy of the "achene" of this species is fundamentally like that of the achene of *Potentilla recta*, with the difference that *Rubus* is bioovulate. Each ventral strand, then, gives rise to an ovule trace.

R. pubescens, *R. flagellaris*, and *R. allegheniensis* are closely similar to *R. hispidus*.

The general situation in all *Rubus* "achenes" is that one strong bundle leaves the stele for each carpel. Very significant exceptions to this, however, are of fairly frequent occurrence, especially in *R. allegheniensis* and *R. flagellaris*. The trace in these cases arises in three parts from one gap. The strands lie very close together and the xylem in some cases is fused, the phloem alone indicating the triple nature of the trace. These three traces, only partly distinct in their origin, fuse completely in the cortex to form a single carpel trace. It is clear that in *Rubus* the single-trace condition has been derived from the three-trace condition and that this has occurred by fusion.

Dalibarda repens

The achene of this species is like that of *R. hispidus*. Three ovule traces instead of the usual two are occasionally found. The first of these is to a suppressed ovule.

Fragaria vesca

The achene of this genus is characterized by the presence of a very weak dorsal bundle. The vascular supply is that of a typical achene. A single trace enters the carpellary base (fig. 23). This trace forks immediately after entering the carpel base, at which level it sends off a very short, very weak bundle (fig. 24*b*). This is the dorsal strand. At a slightly higher level the stronger bundle forks into two equal strands (fig. 25), the ventrals. At a still higher level (fig. 27) one of these gives rise to the ovule trace. Both ventrals continue up the style. The achene of *Fragaria virginiana* is anatomically similar.

Agrimonia striata

In the achene of this species the dorsal bundle has completely disappeared. It never becomes free from the fused ventrals. This achene receives its vascular supply, a single trace, in a manner somewhat different from that of the typical achene. In a typical achene a single trace leaves the stele, moves out through the cortex and into the carpel base. In this species at about the level of carpel trace formation the numerous small bundles in

the receptacle form four groups of vascular tissue (fig. 49). This is followed by the fusion of these groups in pairs (fig. 50); thus there are formed two strong traces, one for each of the two carpels (fig. 51) normally present in a single flower of *Agrimonia striata*. Each carpel, then, receives a single trace. This trace forks only once. The result of this forking is the formation of the two ventral strands (fig. 52). Later one of these ventrals gives rise to the ovule trace. Both ventral strands persist through the style. The dorsal never becomes free from the fused ventrals.

DISCUSSION

The achenes of *Waldsteinia* are unique among those studied in receiving a three-trace supply. Here is an achene very closely related to the follicle in that it has a typical follicle vascular supply, a dorsal and two ventrals. There is no fusion and no reduction of the traces. Each trace enters the carpel base as a single strand and as such moves up the dorsal and the ventral walls into the style. Just as in the follicle, numerous branches from these strands give the ovary wall a very abundant supply of vascular tissue. Each ventral gives rise to an ovule trace; the lower of these, however, is to a suppressed ovule. While achenes of *Waldsteinia* show no reduction in their vascular system, they do show reduction in ovule number as testified by the presence of an ovule trace to a suppressed ovule.

A number of achene-bearing species give anatomical evidence that reduction to the uniovulate condition of the typical achene is not complete. This evidence is in the presence, in some forms, of an ovular trace to a suppressed ovule, as in *Waldsteinia* and *Dalibarda*; and in other forms, of an abortive ovule or ovules without vascular tissue. One to four or five abortive ovules occur regularly in *Anemone*, *Clematis*, *Hepatica*, and some species of *Potentilla*. These ovules range from mere vestiges to structures of fair size. Still further evidence of reduction is found in the ovules of such forms as *Rubus* where, of two ovules, the functional one develops at the expense of the other.

In achenes the persisting functional ovule occupies one of two positions. It is either "basal," as in all of the species of *Ranunculus*, *Geum*, *Duchesnea*, *Fragaria*, and others; or it is "pendulous" as in *Clematis*, *Anemone*, *Hepatica*, *Thalictrum*, *Potentilla*, and others. In the former case it is doubtless the lowermost of the row in the ancestral follicle; in the latter it is the uppermost, or one of the upper few.

With the exception of *Waldsteinia* all the achenes studied by the writer receive a single trace. While this trace is single anatomically, it is not so morphologically. It is clearly a compound trace composed of the two ventrals laterally fused with the dorsal. Plenty of evidence is available to substantiate this statement. The abnormal trace situation found in some carpels of *Helleborus* and *Aquilegia*, where a two-trace and a one-trace supply, respectively, replace the normal three-trace supply, show that in

some follicles reduction of the vascular supply by fusion has already begun, and that in others it has been carried to an advanced stage. The carpellary supply as sometimes found in *Ranunculus Ficaria* (fig. 41) bears out still further the compound nature of the single trace of the achene. Among the many achenes of *R. Ficaria* studied, several show three traces leaving the stele from three gaps. These traces fuse almost immediately in the cortex and enter the carpel base as one trace, clearly a fusion trace made up of three parts which if they had entered the carpel base separately would have become the dorsal and the ventral strands of the carpel. Very soon after entering the carpel base this anatomically single trace splits, forming three equally strong bundles, a dorsal and two ventrals. Carpels are found in *Anemone virginiana*, *Thalictrum polygamum*, *Rubus flagellaris*, and *R. allegheniensis* which show the single entering trace to be undoubtedly a fusion trace composed of three parts. In all of these carpels the three traces, which later fuse in the cortex, leave the stele very close together as if they come from a single gap. Within the carpel base this strand splits, forming two equally strong bundles, a dorsal and a fused ventral. At a higher level the fused ventral forks revealing its double nature. There is, then, in the achene a reduction of vascular tissue. This reduction is accomplished by the fusion of the ventral strands with the dorsal.

The typical vascular anatomy of achenes is as follows: a single trace leaves the stele; within the carpel base this trace splits, forming the dorsal—which, at this level may or may not give rise to two lateral branches—and another bundle, the fused ventrals. At this same level the fused ventrals bundle may or may not fork into its component parts; in the latter eventuality this forking occurs at a varying distance up the ovary wall. In any case the dorsal bears quickly to the dorsal side and so up the ovary wall; the ventral or ventrals, as the case may be, move up the ventral side of the ovary wall. All three strands continue into the style.

Four variations of this general condition will be discussed.

1. The Position of the Ovule Trace

In a follicle, ovule traces definitely depart from the ventral strands; in an achene the point of departure of the ovule trace depends upon the amount of reduction and fusion which has taken place in the ventral strands and the dorsal. One exception to the preceding should be noted, namely, the three-trace achene of *Waldsteinia* (fig. 74), in which there is no fusion of ventrals and dorsal. In this case each ventral gives rise to an ovule trace.

In both the Ranunculaceae and the Rosaceae achenes are found in which the ovule trace definitely departs from a ventral strand—*Ranunculus acris*, *R. Ficaria*, *Anemonella*, *Fragaria*, *Agrimonia*, *Potentilla*, *Rubus*, and *Dalibarda*. In all of these forms the ovule trace clearly proceeds from a ventral above the level at which fusion with the dorsal and with each other ceases. This fusion may cease in the carpel base at the level at which the dorsal and

the two ventrals split from the carpel trace. At a still higher level one of these bundles, clearly a ventral, gives rise to the ovule trace. Such is the condition found in the two species of *Ranunculus* (fig. 59, 60), *Fragaria* (fig. 77), and *Agrimonia* (fig. 78). It is of interest to note that the ovule trace appears at a higher level in *R. acris* (fig. 59) than it does in *R. Ficaria* fig. 60). Fusion of the ventrals with the dorsal towards the persisting ovule has proceeded farther in the case of *R. Ficaria* than in that of *R. acris*. Again this fusion may persist for a varying distance up the ovary wall. In this case it consists of the fused ventrals only, the dorsal having split from the carpel trace soon after the trace enters the carpellary base. As before, one of the two strands formed by the splitting of the fused strand gives rise to the ovule trace above the level at which the splitting occurred. This is the condition found in *Anemonella* (fig. 70), *Potentilla*, *Rubus*, and *Dalibarda* (fig. 77). In *Rubus* and *Dalibarda*, each ventral gives rise to an ovule trace.

In all the remaining species studied in both the Ranunculaceae and the Rosaceae the ovule trace appears simultaneously with the two ventral strands. Reduction of the vascular tissue by fusion has thus progressed to the point where the ventrals are fused up to the point of departure of the ovule trace. This departure may occur in the base of the achene, as in *Ranunculus repens* (fig. 61) or *Geum rivale* (fig. 75), for example, or high up in the ovary wall near the top of the ovular cavity, as in *Anemone canadensis* (fig. 72). In the former case, then, the ovule trace is one of the four bundles formed at the forking of the carpellary trace. It is the median of the upper or ventral group of three strands, and has its origin morphologically in one of the ventral strands, even though this strand is anatomically fused with the dorsal and the other ventral. This, then, is the explanation of the fact that the carpel trace apparently gives rise directly to an ovule bundle. In the case where the ovule trace departs near the top of the ovule cavity, it is one of three bundles, the median, formed at the splitting of the fused ventral, and again has its origin morphologically in one of the ventrals.

Extreme reduction of the ventrals is found in *Ranunculus aquatilis* and *R. longirostris* (fig. 68). So complete is this reduction that the ventrals, as distinct bundles, have completely disappeared; hence the ovule trace appears to come from the dorsal. In the case of these two most reduced species, the strand from which the ovule trace comes is not a simple one composed of the dorsal only; it is a compound one wherein the ventrals are reduced and completely fused with the dorsal. Here, too, the ovule trace comes from a ventral strand morphologically, though apparently from a dorsal.

2. The Dorsal-lateral Strands

Dorsal-lateral strands are found only in certain species of *Ranunculus* (figs. 59, 61, 62, 64), that is, those having a five-veined achene. In no other achenes of either the Ranunculaceae or of the Rosaceae are they present. They are strands which make their appearance at the same level as the

dorsal from whence they arise. They move more or less parallel with it up the dorsal side of the ovary wall. Above the ovular cavity they fuse with the ventrals. The strands which continue beyond this point into the style (fig. 61) may be viewed as either the fused dorsal-laterals and ventrals, or as the ventral bundle only, the dorsal-laterals not extending beyond this level.

R. pennsylvanicus is of particular interest in relation to its dorsal-lateral strands. It represents a transitional form; carpels with full length dorsal-lateral bundles are found along side of those in which these bundles do not appear.

3. The Length of the Ventral Strand

Reduction in the ventral strands may be brought about by fusion with the dorsal strand and with each other, or by an actual shortening in length until the strands disappear as such. The former condition, that is, reduction by fusion, has already been discussed in connection with the departure of the ovule trace.

In the achene, just as in the follicle, a full length ventral extends from the base of the carpel to the base of the stigma. Such is the condition found, for example, in *Ranunculus repens* (fig. 61), *Geum rivale* (fig. 75), and *Rubus allegheniensis*, where the fusion of the dorsal and the ventrals ceases in the carpel base; and in *Thalictrum polygamum* (fig. 71), *Anemone canadensis* (fig. 72), *Rubus flagellaris*, etc., where the fusion of dorsal and ventrals continues well up the ovary wall. When reduction in the length of the ventral strands occurs the stylar portion is the first to drop out. In *Ranunculus acris* (fig. 59) and other species of *Ranunculus* the strands end as such when they anastomose with the dorsal-laterals, approximately at the base of the style; in *Anemone virginiana* (fig. 73) and *Clematis virginiana* they end abruptly at the point where the ovule trace departs; in *Ranunculus Flammula* var. *unalaschensis* (fig. 66), *Duchesnia indica* (fig. 76), and *Potentilla canadensis* they gradually die out part way up the ovary wall. *Ranunculus reptans* (fig. 65) features a change, but no reduction, in the length of the ventrals. After curving over the ovary cavity they fuse with the dorsal. In *R. Cymbalaria* (fig. 67) the ventrals have been reduced to mere stubs; even these stubs have gone in *R. aquatilis* and in *R. longirostris* (fig. 68). This last condition represents complete reduction of the ventrals as such. They never become separated from the dorsal, but remain fused with it throughout their extent.

4. The Length of the Dorsal Strand

Reduction in the length of the dorsal strand may be brought about just as in the ventrals, that is, by fusion with the ventrals and by a decrease in length. Like the ventral it tends to extend from the base of the carpel to the base of the stigma. Such is its length among all the achenes studied in the Ranunculaceae, except in those of *R. longirostris* (fig. 68) where it gradually dies out about half way up the dorsal wall. It is only among the achenes of the Rosaceae that reduction in length of the dorsal has progressed

to the point where it has completely dropped out. A complete series can be found in this family. In *Geum rivale* (fig. 75) and in *Potentilla recta* the dorsal extends from the base of the carpel to the tip of the style; in *Duchesnea* (fig. 76) it ends about one-half way up the dorsal wall; in *Fragaria* (fig. 77) it is reduced to a mere stub; while in *Agrimonia striata* (fig. 78) it is completely suppressed. It never becomes separated from the carpel trace.

The vascular system of several of the achenes of *Ranunculus* described in the preceding pages has been studied by former investigators. With regard to *R. abortivus*, E. A. Bessey (4) says: "The main fibrovascular bundle supplying the pistil divides just after entering it. One branch passes around in the median line in a position corresponding to the bundle of the mid-rib of a leaf. The other passes up into the axillary placenta, through the funiculus and into the base of the nucellus also in the median line. Later other branches run to the side walls of the pistil." Bessey recognized the dorsal and the ovule strands, but evidently found no ventral strands. Smith (17) reports finding only mere stubs for the ventral traces in this species. The present investigator finds well formed and definite ventral traces curving over the ovular cavity.

There are discrepancies also between the accounts of Smith (17) and those of the writer for *R. hispidus*, but these are largely due to a difference in interpretation of the strands present.

G. Henslow (12), in his description of *R. Flammula*, a species not studied by the writer, writes as follows: the "cord which supplies the carpel divides into two branches, one dorsal and a marginal or placental one. The latter supplies the 'basal ovule,' then passes over the ovary and curls backwards, finally joining the dorsal." In all the species of *Ranunculus* worked over by the writer, except the reduced aquatic plants, *R. aquatilis* and *R. longirostris*, the ventral (Henslow's marginal) always forks into two definite strands either before or simultaneously with the departure of the trace to the ovule. Apparently Henslow's interest in this species lay in the unusual condition of a marginal strand anastomosing with the dorsal.

CONCLUSIONS

1. There is anatomical and morphological evidence that the achene is a reduced follicle. This evidence consists in proof that the number of ovules has been reduced from several to one, and that the simple vascular supply of achenes has been reduced from the more complex supply of follicles.

2. The evidence concerning ovules consists in the presence, in some forms, of an ovular trace to a suppressed ovule; in other forms, of abortive ovules without vascular tissue; and in still others, of rudimentary ovules.

3. The evidence concerning vascular tissues consists in series of pistils in the Ranunculaceae and Rosaceae which show all stages of reduction from the typical three-trace follicle to the most simple one-trace achene. Reduction of the vascular tissue has clearly steadily accompanied the reduction in number of ovules, and in size of carpel.

4. The single trace of an achene is not a simple trace but a compound one formed by the lateral fusion of the two ventral and the dorsal traces.

5. The two extra strands, the dorsal-laterals, which appear in the dorsal wall of some of the larger carpels of *Ranunculus* giving the carpel a five-strand venation, are branches of the dorsal which appear at the very base of this trace. In the reduction of vascular tissue which occurs in the evolution of the achene they are the first strands to disappear.

6. The reduction of the vascular system within the achene itself accompanies and follows a fusion of the ventral bundles with each other and with the dorsal. The greater the fusion of the ventrals with the dorsal, the closer to this point of fusion is the level of departure of the ovule trace; where the fusion of the three bundles extends to the point of departure of the ovule trace, the trace departs, as it were, from the carpel trace itself.

7. The achenes of the Rosaceae do not show the advanced reduction in vascular tissue present in those of the Ranunculaceae. One genus, *Waldsteinia*, has an achene which receives the same vascular supply as a follicle, namely, a dorsal and two ventrals. There are no achenes without ventral traces.

8. Within the achene of the Rosaceae there has been a greater reduction in the dorsal bundle than in the ventral bundles. When this reduction is greatest the dorsal never becomes free from the fused ventrals. This contrasts with the condition in *Ranunculus* where, in great reduction, the ventrals are fused with the dorsal throughout their length.

9. Amongst the Ranunculaceae the most advanced reduction of vascular tissue is found within the achenes of *Ranunculus*. There is a more or less gradual shortening of the ventral bundles until no free parts remain; the ovule then seems to depart from the dorsal. Reduction has progressed in the ventrals until ultimately it has resulted in the complete disappearance of these bundles. The dorsal shows only a slight reduction, a shortening in some species. This contrasts with the condition in *Agrimonia* where, in great reduction, the dorsal never becomes separated from the ventrals.

The writer wishes to express her appreciation of the advice and kindly criticism of Professor Arthur J. Eames of Cornell University, and for his suggestion of this problem.

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EXPLANATION OF PLATES

All figures are purely diagrammatic. The following symbols are used: *a*, ventral strand; *b*, dorsal strand; *c*, carpel trace; *d*, ovule trace; *e*, dorsal-lateral strand; dashes indicate a strand not in the plane of section of the diagram; dots, a strand which may or may not be present; crossed lines fused bundles.

PLATE XLII

FIGS. 1-3. *Aquilegia canadensis*. Diagrams of cross sections of the axis: 1. At level of departure of dorsal trace (*b*) and the last stamen traces (*f*). 2. At level of departure of ventral traces (*a*). 3. Diagram of cross section of a carpel through its base showing the three-trace supply of the carpel.

FIG. 4. *Delphinium cultorum*. Diagrams of a cross section through the axis at the level of formation of carpellary vascular supply.

FIGS. 5-7. *Helleborus viridis*. Diagrams of cross sections of two carpels, *x* normal, *y* abnormal, at successively higher levels.

FIGS. 8-15. *Thalictrum polygamum*. 8. A diagram of a longitudinal section of an achene showing part of its vascular tissue and the pendulous ovule. 9-15. Diagrams of cross sections of a carpel at successively higher levels. 9. A diagram showing the composite structure of the apparently single carpel trace, and six ridges supplied with bundles. 10. All eight ridges supplied with bundles. 11. The bundles from the two ridges on either side of the fused ventral (*a*) begin to move in towards it. 12-13. The fused ventral splits forming the two ventrals (*a*) and the ovule trace (*d*). 14. The fusion of the ventrals with the bundle from the nearest rib; the carpel is now supplied with seven bundles instead of

eight as in fig. 10. 15. Cross section through the style showing the three persisting bundles, probably the two ventrals and the dorsal.

FIGS. 16-21. *Anemonella thalictroides*. Diagrams of cross sections through the upper part of a carpel at successively higher levels showing: 16. Each of the eight ridges of the carpel with its own vascular bundle. 17. The splitting of the fused ventral into the two ventrals. 18. Bundles from the two nearest ridges moving in towards the ventrals. 19. One ventral giving rise to the ovule trace. 20. The ovule trace passing into the ovule, and the fusion of the ventrals with bundles from the nearest ridges. 21. The seven bundles which continue up the ovary wall.

FIG. 22. *Anemone canadensis*. Diagram of a longitudinal section of a carpel showing its vascular system, pendulous ovule, and rudimentary ovules (x).

FIGS. 23-28. *Fragaria vesca*. Diagrams of cross sections of a carpel at successively higher levels showing: 23. The carpel trace (c); 24. The passing off of the dorsal strand (b); 25-26. The formation of the two ventral strands; 27-28. The origin and passing off of the ovule trace.

PLATE XLIII

FIGS. 29-40. *Ranunculus acris*. 29. A diagram of a longitudinal section of an achene showing its vascular system (only one ventral in plane of section) and the basal ovule. 30-40. Diagrams of cross sections at the levels a-a', b-b', etc.

FIG. 41. *Ranunculus Ficaria*. A diagram of a cross section of a carpel showing the composite nature of the single carpellary trace.

FIGS. 42-48. Diagrams representing a longitudinal section of an achene showing vascular structure and the basal ovule in the following: FIG. 42. *Ranunculus Ficaria*; FIG. 43. *Ranunculus repens*; FIG. 44. *Ranunculus pennsylvanica*; FIG. 45. *Ranunculus reptans*; FIG. 46. *Ranunculus longirostris*; FIG. 47. *Ranunculus Flammula* var. *unala-schensis*; FIG. 48. *Ranunculus Cymbalaria*.

FIGS. 49-52. *Agrimonia striata*. 49-50. Diagrams of cross sections of axis just below level of carpel formation. 49. All of vascular tissue remaining in axis forms four groups. 50. These groups fuse in pairs. 51. Cross sections of a pistil showing perigynium (k) surrounding the two carpels each containing only one vascular bundle, the fused ventrals. 52. One of the carpels of fig. 51, the fused ventral, has split into the two ventrals (a).

PLATE XLIV

A series of diagrams representing the vascular supply of carpels; its reduction by fusion and by the shortening of the bundles; the number and position of ovules in follicles (figs. 53-58) and in achenes (59-79). It also shows the derivation of the uniovulate single-trace carpel (achene) from the multiovulate three-trace carpel (follicle).

FIG. 53. *Helleborus viridis*.

FIG. 54. *Aquilegia canadensis*.

FIG. 55. *Hydrastis canadensis*.

FIG. 56. *Isopyrum*.

FIG. 57. *Helleborus viridis* (unusual).

FIG. 58. *Aquilegia canadensis* (unusual) (after Smith, 17).

FIG. 59. *Ranunculus acris*.

FIG. 60. *Ranunculus Ficaria*.

FIG. 61. *Ranunculus repens*.

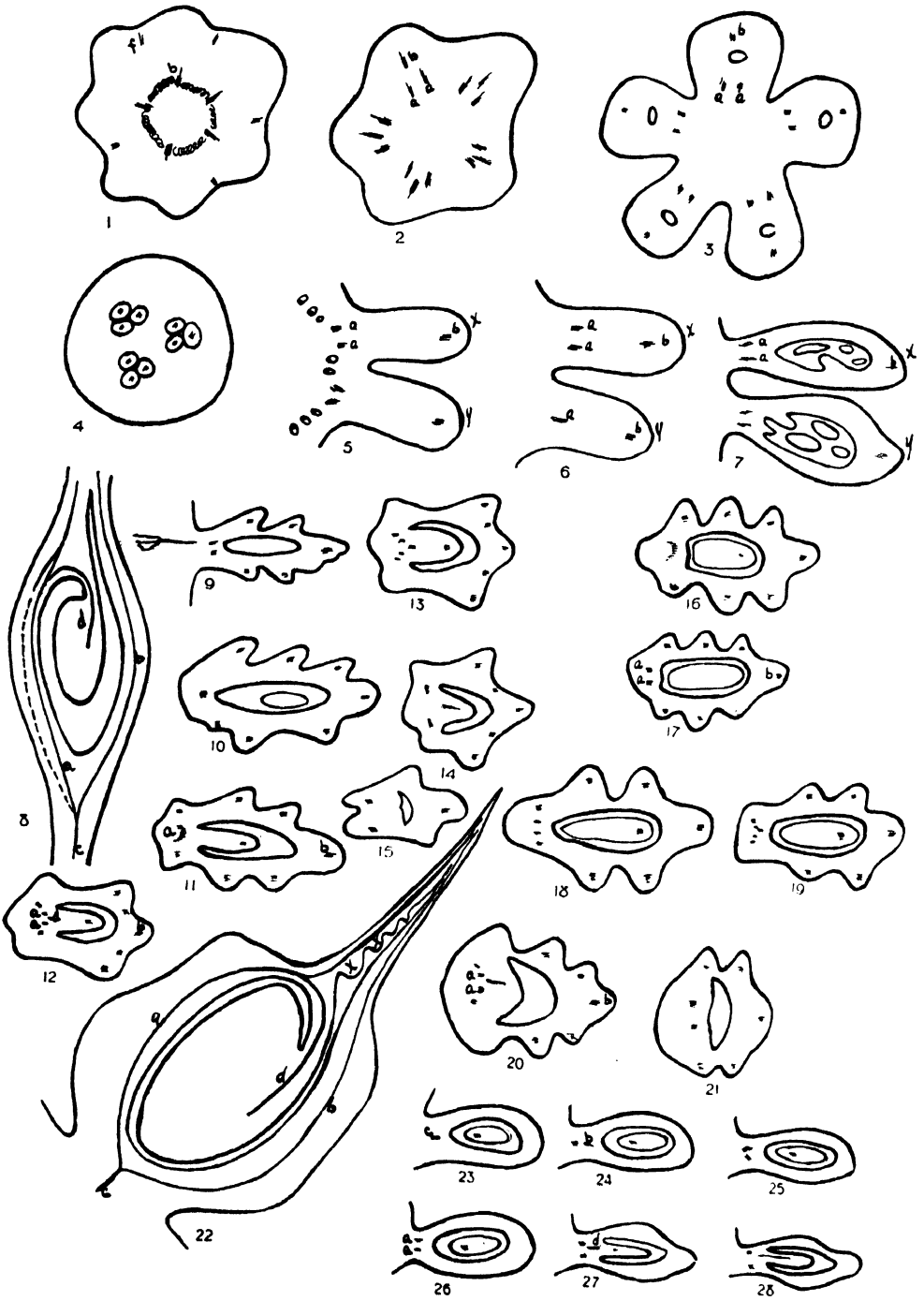
FIG. 62. *Ranunculus hispidus*.

FIG. 63. *Ranunculus recurvatus*.

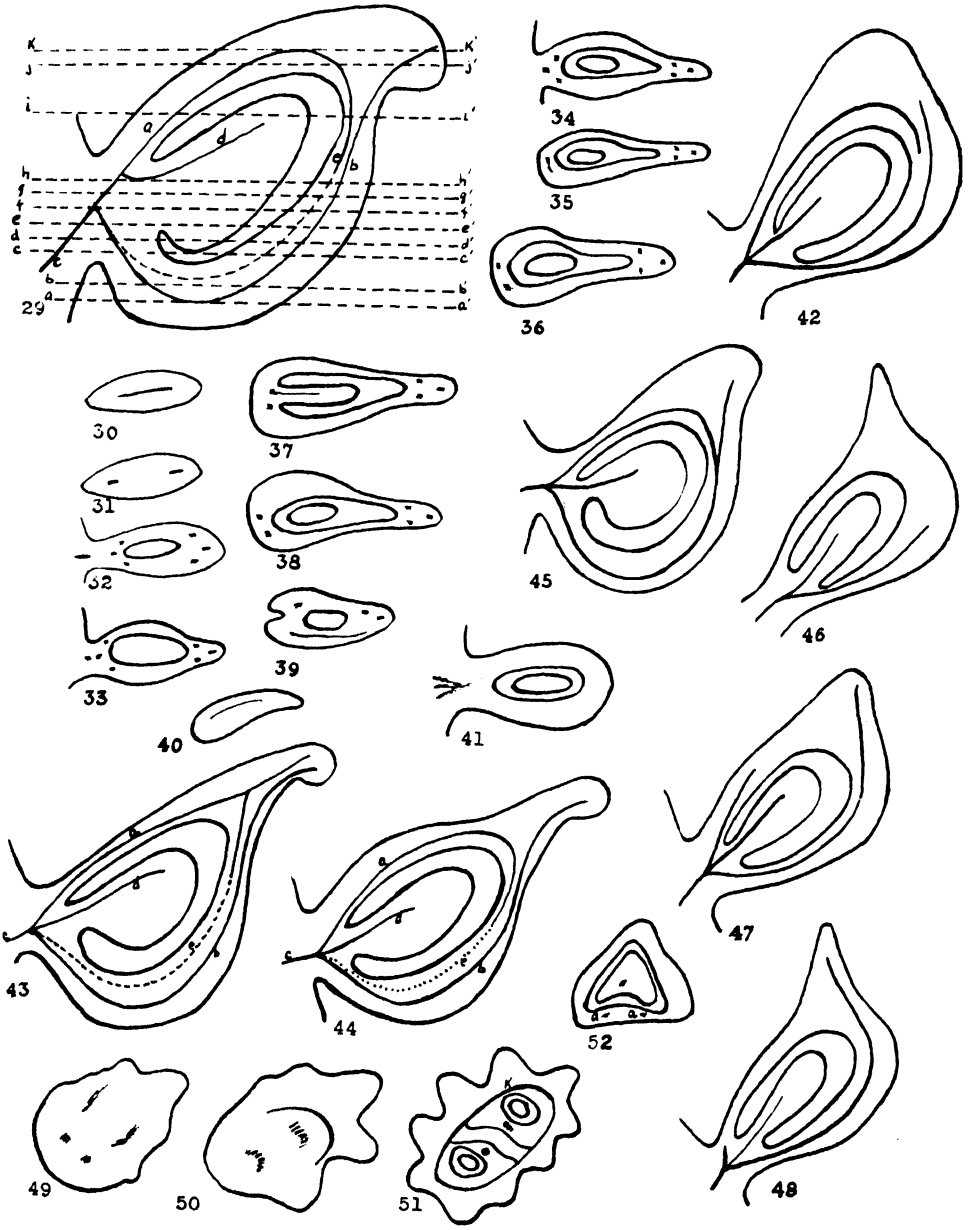
FIG. 64. *Ranunculus pennsylvanicus*.

FIG. 65. *Ranunculus reptans*.

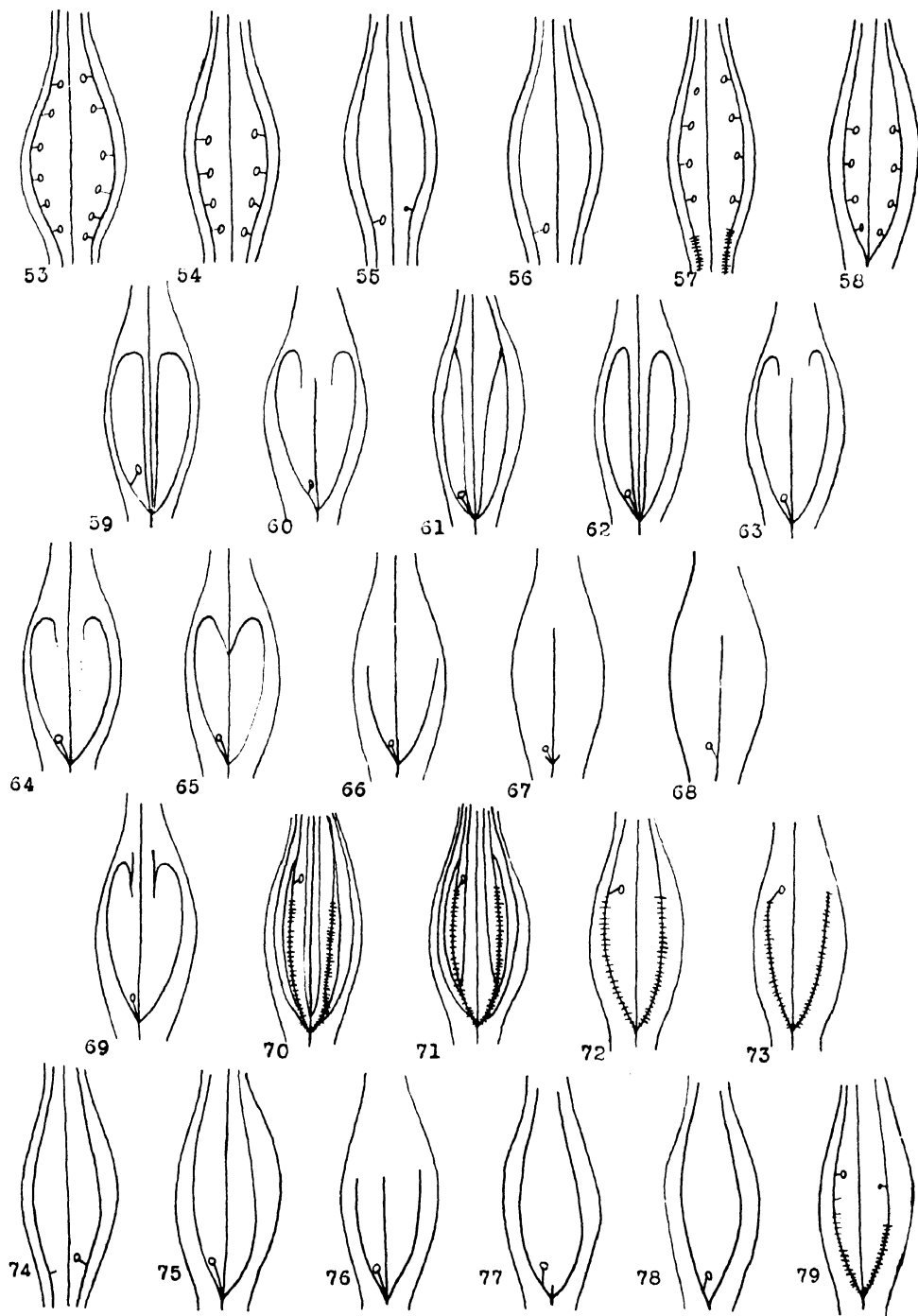
FIG. 66. *Ranunculus Flammula*.



CHUTE: ACHENE MORPHOLOGY



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- FIG. 67. *Ranunculus Cymbalaria*.
- FIG. 68. *Ranunculus longirostris*.
- FIG. 69. *Myosurus minimus*.
- FIG. 70. *Anemonella thalictroides*.
- FIG. 71. *Thalictrum polygamum*.
- FIG. 72. *Anemone canadensis*.
- FIG. 73. *Anemone virginiana*.
- FIG. 74. *Waldsteinia fragarioides*.
- FIG. 75. *Geum rivale*.
- FIG. 76. *Duchesnea indica*.
- FIG. 77. *Fragaria vesca*.
- FIG. 78. *Agrimonia striata*.
- FIG. 79. *Dalibarda repens*.

COMPARATIVE ANATOMY OF THE WOODS OF THE MELIACEAE¹

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INTRODUCTION

A study of the correlation between the gross morphological and anatomical characters of certain families has as yet received little consideration. Systematic botanists have disregarded almost entirely the anatomical characters of the stem in their present schemes of classification. This may be due to the fact that wood samples are rather difficult to obtain and prepare for study, or to the general consensus of opinion that no correlation exists between morphological and anatomical characters. There is no doubt, however, that anatomical study will bring to light many important facts which will be of value to the systematic botanist in clearing up doubtful points. In the study of the comparative anatomy of the woods of the Juglandaceae by the author (9), for example, several facts of this sort were discovered, namely: (1) that the woods of *Apocarya* and *Eucarya* are easily separable; (2) that the woods of *Juglans* are divided into two distinct groups which correspond with the two groups separated by the structure of the nut; (3) that there is a difference in wood structure between the two sections *Oreomunnea* and *Pterilema* of the genus *Engelhardtia*; and (4) that *Alfaroa* belongs with the *Oreomunnea* section of *Engelhardtia*.

Similar examples could be cited from other families. In cases where investigators have been unable to find anatomical characters of taxonomic value they have usually either been working with groups which were more or less artificial and heterogeneous (like the Magnoliaceae in the earlier sense) or have endeavored to use for diagnostic purposes single isolated characters rather than a particular combination of characters.

The present investigation was undertaken in an endeavor to throw light on the classification of the Meliaceae by a study of the comparative anatomy of the wood of its species, supplementing data from this source by facts from gross morphology. One hundred and twelve species of the family, representing 36 genera, were studied.

CHARACTERISTICS OF THE MELIACEAE ON THE BASIS OF GROSS MORPHOLOGY

Trees or shrubs. Leaves alternate, exstipulate, mostly pinnate, rarely simple or bi-pinnate; leaflets opposite or alternate. Flowers actinomorphic,

¹ The greater part of this investigation was carried on while the author was the holder of a Sterling Fellowship in botany at Yale University.

mostly ♂, usually in axillary panicles or racemes. Calyx 3-6-lobed, sometimes with free sepals, usually imbricate. Petals 3-6, free or partially connate, contorted or imbricate, or adnate to the staminal-tube and valvate. Stamens 3-12; filaments connate or rarely free; anthers erect, usually sessile on the tube, included or exserted, 2-celled, dehiscing longitudinally. Disk various. Ovary superior, 2-5-celled; ovules 2-14 in each cell, rarely 1, collateral or superposed. Stigma disciform or capitate. Fruit capsular, drupaceous, or baccate. Seeds albuminous or exalbuminous, sometimes winged.

Distribution: About 40 genera and 1217 species, mostly tropical or subtropical.

CHARACTERISTICS OF THE MELIACEAE ON THE BASIS OF WOOD STRUCTURE

Gross Structure

Color creamy white to dark chocolate brown, mostly red or reddish-brown; luster high or dull. Odor aromatic or not distinctive. Soft to very hard, sp. gr. (oven-dry), 0.37 to 1.24. Straight to decidedly roey-grained; fine to coarse-textured.

Growth rings distinct to apparently absent. Wood parenchyma conspicuous to not visible with lens; distribution paratracheal, as isolated cells to 3 cells wide; terminal in rather even concentric lines 3 to 10 mm. apart; or metatracheal either in closely spaced broken to continuous tangential lines 3 to 9 per mm., or diffuse as scattered cells. One genus is distinctly ring-porous; one ring-porous to diffuse-porous; the others diffuse-porous. The pores vary from not visible with lens to plainly visible as small to large pin holes. Vessel lines vary from indistinct to distinct as coarse scratches. The rays vary from not visible without lens to distinct on the cross and tangential sections; inconspicuous to conspicuous on the radial producing a distinct silver grain. Ripple marks absent to distinct. Vertical traumatic gum ducts present or absent.

Minute Anatomy

The pores range from minute to large and occur solitary or in radially appressed groups of 2 to 8; the lumina in the majority of woods are plugged with a yellowish or reddish gum. Vessels with small, oval, simple perforations; intervacular pits mostly minute, but in some genera large; they are numerous, alternately arranged, with oval to polygonal borders and lenticular apertures which frequently coalesce to form false spirals. Fibers thin to very thick-walled; lumina free of contents or plugged with red gum; septations present or absent; pits simple or bordered. The rays vary from homogeneous to decidedly heterogeneous, from 1 to 10 cells wide, and from 2 to 150 cells high; crystals in the marginal cells are present in the Swietenioideae; lumina usually plugged with a red or yellowish gum. Vessel-ray pits of the same type as the intervacular. Crystals in the wood parenchyma are present or absent.

A NATURAL KEY TO THE GENERA ON THE BASIS OF MORPHOLOGICAL CHARACTERS²

- I. Ovary with from 6-14 ovules in each cell; staminal-tube 8-10-toothed; (stamens free in *Cedrela*); flowers 5-merous, or 4-5-merous; seeds numerous, winged, attached to column by seed (not winged in *Carapa*, *Xylocarpus*); capsule 3-5-celled, dehiscing into 3-5 woody valves. **Swietenioideae**
1. Stamens free; seeds albuminous. *Cedrela*
 2. Stamens united to form staminal-tube.
 - A. Seeds not winged, very large, pyramid-shaped; ovary with 6-8 ovules in each cell.
 - a. Inflorescence few-flowered; staminal-tube teeth bipartite; leaves with 1-3 pairs of leaflets; capsule with cork-like husk. *Xylocarpus*
 - b. Inflorescence many-flowered; staminal-tube teeth entire; leaves with many pairs of leaflets; capsule with woody-husk. *Carapa*
 - B. Seeds winged; ovary with 8-14 ovules in each cell.
 1. Seeds winged all around. *Khaya*
 2. Seeds winged above. *Swietenia*
 3. Seeds winged below.
 - a. Ovary 5-celled; capsule 5-celled, dehiscing into 5 valves.
 - a¹. Disk cupular; staminal-tube not partitioned; valves of capsule connected by fibrous network after dehiscence; leaflets undulately toothed. *Pseudocedrela*
 - b¹. Disk stipitiform; lower part of staminal-tube partitioned; valves of capsule not connected by fibrous network; leaflets entire. *Entandrophragma*
 - b. Ovary 3-celled; capsule 3-celled, dehiscing into 3 valves. *Chickrassia*
 4. Seeds winged above and below. *Soyimida*

II. Ovary with 4 ovules in each cell; staminal-tube 8-toothed; flowers 4-merous; seeds 4, attached to column by end of wing; capsule 4-celled, dehiscing into 4 membraneous valves. **Lovoinoideae** *Lovoa*

III. Ovary with 1-2 ovules in each cell; stamens united or partly united to form staminal-tube; seeds not winged. **Melioideae**

 - A. Leaves 2-3-pinnate; leaflets toothed; fruits drupaceous. *Melia*
 - B. Leaves simple pinnate or trifoliolate.
 - I. Fruit a capsule; seeds exalbuminous.
 - a. Capsule coriaceous; ovary more than one-celled.
 - a¹. Disk o, annular, or confluent with ovary.
 - a². Anthers exerted; stamens frequently united at base only or teeth of tube bi-partite.
 - a³. Capsule 1-celled with one seed, dehiscent into 2 valves. *Heynea*
 - b³. Capsule 2-3-celled with 1-2 seeds in each cell, dehiscent into 2-4 valves. *Trichilia*

² This key was derived from the gross morphological descriptions as given by the following authors: Aubreville (1); Benthams, G. (2); Benthams and Hooker (3); Brandis (4); De Candolle (5); Engler and Prantl (6); Hutchinson and Dalziel (7); King (8); Oliver (10); Parker (11); Perkins (12); Phillips (13); Ridley (14); Standley (16).

- b². Anthers included; stamens united to apex to form staminal-tube; teeth not bi-partite.
 - a³. Capsule 3-celled, dehiscent, usually into 3 valves.
 - a⁴. Petals 3.
 - a⁵. Flowers dioecious; anthers 3-6; stigma small, sessile *Aphanamixis*
 - b⁵. Flowers monoecious; anthers 6-10; stigma large, conical *Amoora*
 - b⁴. Petals 4, rarely 5 *Synoum*
 - b³. Capsule 4-5-celled, dehiscing into 4-5 valves *Guarea*
 - b¹. Disk free, tube-like or cylindric.
 - a². Ovary 2-4-celled with one ovule in each cell; stigma capitate *Chisocheton*
 - b². Ovary 4-5-celled with 2 ovules in each cell; stigma discoid.
 - a³. Capsule dehiscent *Dysoxylum*
 - b³. Capsule indehiscent *Cabrlea*
 - b. Capsule fleshy; ovary one-celled *Turreanthus*
- II. Fruit berry-like, seed-like, or drupaceous.
- 1. Fruit berry-like.
 - a. Stamens united at base, free at top.
 - a¹. Seeds albuminous; filaments 2-toothed at apex *Cipadessa*
 - b¹. Seeds exalbuminous; filaments not 2-toothed *Walsura*
 - b. Stamens united to apex to form staminal-tube.
 - a¹. Anthers in two rows *Lansium*
 - b¹. Anthers in one row.
 - a². Berry fleshy; stigmas 5 *Sandoricum*
 - b². Berry coriaceous; stigma one.
 - a³. Anthers included; ovary 1-3-celled *Aglaiia*
 - b³. Anthers exerted; ovary 4-5-celled *Ekebergia*
 - 2. Fruit drupaceous; anthers exerted *Owenia*
 - 3. Fruit seed-like, a single woody endocarp *Azadirachta*

C. Leaves simple.

 - a. Stamens united at base, free above, 5 short, 5 long *Vavaea*
 - b. Stamens united to apex to form staminal-tube.
 - a¹. Anthers in 2 rows, appendiculate *Reinwardtiendendron*
 - b¹. Anthers in one row, not appendiculate.
 - a². Ovary 5-10-20-celled; anthers included; stigma discoid *Turraea*
 - b². Ovary 4-5-celled; anthers exerted; stigma capitate *Quivisia*

A NATURAL KEY TO THE GENERA ON THE BASIS OF WOOD STRUCTURE

- I. Crystals in marginal ray cells common; (scarce in *Chickrassia*); rays heterogeneous; terminal wood parenchyma in regular to irregular concentric lines 2 to 10 mm. apart (mostly absent in *Khaya*); crystals in wood parenchyma rare, when present, solitary (forming short chains in *Chickrassia*); fibers septate with simple pits (partly septate in *Cedrela* and *Chickrassia*); intervacular pits minute, 0.003 mm. diam. (exception, *Cedrela*); wood red, reddish-brown, or russet **Swietenioideae**

- A. Terminal wood parenchyma in regular to irregular concentric lines 2 to 10 mm. apart.**
- a. Wood ring-porous.** *Cedrela*
 - b. Wood diffuse-porous.**
 - a¹. Fibers septate; wood without distinctive odor.**
 - a². Metatracheal wood parenchyma in numerous closely spaced tangential lines connecting with or independant of pores; gum ducts absent.**
 - a³. Wood parenchyma conspicuous on all sections; wood very hard, sp. gr. 1.06-1.18; dark chocolate brown; fibers extremely thick-walled; mucilaginous layers common; rays 1-10 cells wide, mostly 5-6, up to 150 cells high; ripple marks absent.** *Soyimida*
 - b³. Wood parenchyma visible but not conspicuous on any section; wood moderately hard, sp. gr. 0.43-0.63; light to dark reddish-brown; fibers thin to thick-walled; mucilaginous layers absent; rays 1-5 cells wide, mostly 3-4, up to 50 cells high; ripple marks present or absent, when present, of local occurrence and irregular.** *Entandrophragma*
 - b². Metatracheal wood parenchyma not in tangential lines; diffuse, rather scarce; gum ducts present or absent.**
 - a³. Pores barely visible without lens; rays barely visible on cross-section; inconspicuous on the radial; ripple marks always distinct; wood dark red.** *Xylocarpus*
 - b³. Pores distinct as small to large pinholes; rays distinct on all sections and produce a silver grain on the radial; ripple marks distinct, irregular, or absent.**
 - a⁴. Wood light reddish-brown to chocolate, without distinct golden luster; grain mostly straight; pores without whitish substance; ripple marks absent.** *Carapa*
 - b⁴. Wood light red to dark reddish-brown with a distinct golden luster; grain mostly roey producing a ribbon effect; pores plugged with whitish substance common.**
 - a⁵. Rays 1-5 cells wide, mostly 3-4; ripple marks when present, distinct.** *Swietenia*
 - b⁵. Rays 1-8 cells wide, mostly 5-6; ripple marks when present, irregular.** *Pseudocedrela, Khaya*¹
 - b¹. Fibers partly septate, sparingly developed; wood with aromatic odor.**
 - a². Wood light pinkish-red to dark red; soft to moderately hard, sp. gr. 0.30-0.64; rays distinct on cross-section, conspicuous on radial; intervacular pits large, 0.006 mm. diam.** *Cedrela*

¹ Well defined terminal parenchyma has been observed in one authentic specimen of *Khaya senegalensis* A. Juss.

- b². Wood olive-brown or russet, somewhat variegated; moderately hard to hard, sp. gr. 0.56–0.80; rays not visible on the cross-section without lens; inconspicuous on radial; intervacular pits minute, 0.003 mm. diam. *Chickrassia*
- B. Terminal wood parenchyma absent; parenchyma diffuse, scarce, not visible with lens. *Khaya*
- II. Crystals in marginal ray cells absent; rays various; wood parenchyma chambered and containing crystals in long chains (exceptions, *Turraea*, *Quivisia*, *Aphanamixis*, *Cabralea*); fibers with bordered pits. **Melioidae**
- A. Wood ring-porous; minute vessels in clusters forming wavy tangential bands; vessels with spirals; wood light brown to red. *Melia*
- B. Wood diffuse-porous; minute vessels absent, or not forming wavy tangential bands.
1. Wood parenchyma *terminal* in regular to irregular concentric lines 2 to 10 mm. apart.
- a. Vessels with spirals; minute vessels present; wood light brown to dark red. *Azadirachta*
- b. Vessels without spirals; minute vessels absent.
- a¹. Fibers septate; rays uniseriate; wood light red. . . . *Cipadessa*
- b¹. Fibers non-septate; rays multiseriate in part.
- a². Rays homogeneous; intervacular pits large, 0.0065 mm. diam.; wood light yellowish-brown; gum ducts absent. *Ekebergia*
- b². Rays heterogeneous; intervacular pits minute, 0.003 mm. diam.; wood greyish-pink or dark red; gum ducts present or absent.
- a³. Wood soft, greyish-pink; fibers thin-walled, free of contents; rays 1–3 cells wide. . . *Sandoricum*
- b³. Wood hard, dark red; fibers thick-walled, lumina filled with red gum; rays 1–4 cells wide. *Ocotea*
2. Wood parenchyma *metatracheal* in continuous or broken tangential lines 3 to 9 per mm.
- a. Rays homogeneous.
- a¹. Fibers non-septate; rays 1–4 cells wide; color variegated, light to dark reddish-brown with purple; wood parenchyma conspicuous. *Walsura*
- b¹. Fibers septate; rays 1–2 cells wide.
- a². Wood light yellowish-brown with golden luster; odor aromatic; hard, sp. gr. 0.82–0.98; concentric lines of wood parenchyma with crystals; fibers very thick-walled; mucilaginous layers common. *Reinwardtiodendron*
- b². Wood light to dark red without golden luster; aromatic odor present or absent; soft to moderately hard, sp. gr. 0.47–0.75; concentric lines of wood parenchyma with crystals absent; fibers with thin to thick walls; mucilaginous layers absent. *Guarea*
- b. Rays heterogeneous.

- a¹. Fibers non-septate.
 - a². Rays uniseriate (1-2 cells wide in *T. emetica* Vahl. and *T. lanata* A. Chev.); wood light reddish-brown or orange brown *Trichilia*
 - b². Rays 1-4 cells wide, mostly triseriate; wood light to dark grey *Heynea*
 - b¹. Fibers septate.
 - a². Wood parenchyma chambered with crystals in long chains.
 - a³. Wood dark red or dark reddish-brown.
 - a⁴. Multiseriate rays exclusively biseriate.
 - a⁵. Rays not visible without lens on the cross-section; wood parenchyma not conspicuous on any section; intervacular pits minute, 0.003 mm. diam.; wood hard with aromatic odor, sp. gr. 0.76-1.10 *Aglaia*
 - b⁵. Rays barely visible without lens on cross-section; wood parenchyma conspicuous; intervacular pits large, 0.006 mm. diam.; wood without distinctive odor, sp. gr. 0.57-0.73 *Synoum*
 - b⁴. Multiseriate rays up to 3 cells wide; wood parenchyma conspicuous; rays not visible on cross-section without lens *Dysoxylum*
 - b³. Wood light yellow, light yellowish-brown, or light pink; wood parenchyma conspicuous.
 - a⁴. Wood light yellowish-brown, hard, sp. gr. 0.71-0.90 *Lansium*
 - b⁴. Wood cream color or light pink, moderately hard, sp. gr. 0.56-0.69 *Chisocheton*
 - b². Wood parenchyma without crystals.
 - a³. Multiseriate rays 1-2 cells wide mostly uniseriate; wood parenchyma not conspicuous, barely visible without lens; intervacular pits large, 0.006 mm. diam.; wood light to dark red *Aphanamixis*
 - b³. Multiseriate rays 1-3 cells wide, mostly biseriate; wood parenchyma conspicuous on all sections; intervacular pits minute, 0.003 mm. diam.; wood very dark red or chocolate *Cabralea*
3. Wood parenchyma *diffuse*, scarce, not visible with lens; rays heterogeneous; wood cream color, yellow, or red.
- a. Fibers non-septate; rays with uniseriate tips several times the length of the multiseriate portion; wood parenchyma without crystals; pores not visible without lens; intervacular pits apparently with cribriform membranes; wood yellow with a brownish tinge *Turraea, Quivisia*
 - b. Fibers septate; rays with uniseriate tips short or absent; wood parenchyma with crystals.

- a¹. Pores minute, barely visible without lens; intervacular pits apparently with cribriform membranes; wood cream color *Vavaea*
- b¹. Pores medium to large, distinct without lens; intervacular pits without cribriform membranes.
 - a². Wood red; lumina of vessels and fibers plugged with red gum *Amoora*
 - b². Wood cream color; lumina of vessels filled with yellow gum; fibers free of contents *Turreanthus*
- III. Crystals in marginal ray cells absent; wood parenchyma scarce, occurs diffuse and paratracheal characteristically on one side of pore and occasionally extends out in short tangential lines; not visible with lens; lumina contains numerous crystals in long chains; rays homogeneous; fibers non-septate with simple pits; intervacular pits minute, 0.003 mm.; wood dark greyish-brown **Lovoinoideae** *Lovoa*

AN ARTIFICIAL KEY TO THE GENERA ON THE BASIS OF WOOD STRUCTURE

- A. Ring-porous, *i.e.*, with the largest pores in a distinct zone.
 - a. *Minute* pores associated with larger pores or in large clusters forming wavy tangential bands; small vessels with spirals; fibers with bordered pits; woods without distinctive odor (aromatic in *M. Azadirachta*) *Melia*
 - b. *Minute* pores absent; vessels without spirals; fibers with simple pits; woods with distinct aromatic odor *Cedrela*
- B. Diffuse-porous, *i.e.*, without definite zonate arrangement of pores.
 - I. Wood parenchyma diffuse, scarce, normally not visible with lens.
 - 1. Rays homogeneous; wood parenchyma chambered with crystals in long chains; fibers with simple pits; wood dark olive-brown *Lovoa*
 - 2. Rays heterogeneous.
 - a. Fibers non-septate; rays with uniseriate tips several times the length of the multiseriate portion; wood parenchyma without crystals; fibers with bordered pits; pores not visible without lens; intervacular pits apparently with cribriform membranes; wood dark yellow *Turraea, Quivisia*
 - b. Fibers septate; rays with uniseriate tips short or absent.
 - a¹. Crystals present in marginal ray cells; wood parenchyma without crystals, or if present, solitary; rays 1-8 cells wide, mostly 5-6; ripple marks present or absent; fibers with simple pits; gum ducts present or absent; whitish substance in vessels common; wood light to dark red or reddish-brown with golden luster *Khaya*
 - b¹. Crystals in rays absent; wood parenchyma chambered and containing crystals in long chains; rays 1-3 cells wide; ripple marks absent; gum ducts absent; fibers with bordered pits; wood without golden luster.

- a². Pores minute, barely visible without lens; intervacular pits apparently with cribriform membranes; wood cream color. *Vavaea*
 - b². Pores medium to large, distinct without lens; intervacular pits without cribriform membranes.
 - a³. Wood red; lumina of vessels and fibers plugged with red gum. *Amoora*
 - b³. Wood cream color; lumina of vessels filled with yellow gum; fibers free of contents. . . . *Turreanthus*
- II. Wood parenchyma in concentric or broken tangential lines.
 - A. Terminal parenchyma in regular to irregular concentric lines 2 to 10 mm. apart.
 - i. Crystals in marginal ray cells common; crystals in wood parenchyma rare, when present, solitary; fibers with simple pits; rays heterogeneous.
 - a. Fibers septate; intervacular pits minute, 0.003 mm. diam.; wood without distinctive odor.
 - a¹. Metatracheal wood parenchyma in numerous closely spaced tangential lines connecting with or independent of pores; gum ducts absent.
 - a². Wood parenchyma conspicuous on all sections; wood very hard, sp. gr. 1.06-1.18, dark chocolate brown; fibers extremely thick-walled; mucilaginous layers common; rays 1-10 cells wide, mostly 5-6, up to 150 cells high; ripple marks absent. *Soyimida*
 - b². Wood parenchyma visible but not conspicuous on any section; wood moderately hard, sp. gr. 0.43-0.63, light to dark reddish-brown; fibers thin to thick-walled; mucilaginous layers absent; rays 1-5 cells wide, mostly 3-4, up to 50 cells high; ripple marks present or absent, when present, of local occurrence and irregular. *Entandrophragma*
 - b¹. Metatracheal wood parenchyma not in tangential lines; occurs diffuse, rather scarce; gum ducts present or absent.
 - a². Pores barely visible without lens; rays barely visible on cross-section; inconspicuous on the radial; ripple marks always distinct; wood dark red. *Xylocarpus*
 - b². Pores distinct as small to large pinholes; rays distinct on all sections and produce a silver grain on the radial; ripple marks distinct, irregular, or absent.
 - a³. Wood light reddish-brown to chocolate, without distinct golden luster; grain mostly straight; pores without whitish substance; ripple marks absent. *Carapa*
 - b³. Wood light red to dark reddish-brown with a distinct golden luster; grain

- mostly roey producing a ribbon effect; pores plugged with whitish substance common.
- a⁴. Rays 1-5 cells wide, mostly 3-4; ripple marks when present, distinct... *Swietenia*
- b⁴. Rays 1-8 cells wide, mostly 5-6; ripple marks when present, irregular... *Pseudocedrela*,⁴ *Khaya*
- b. Fibers partly septate, sparingly developed; wood with aromatic odor.
- a¹. Wood light pinkish-red to dark red or reddish-brown; soft to moderately hard, sp. gr. 0.30-0.64; rays distinct on cross-section, conspicuous on radial; crystals in marginal cells common; intervacular pits large, 0.006 mm. diam. *Cedrela*
- b¹. Wood olive-brown or russet, somewhat variegated; moderately hard to hard, sp. gr. 0.56-0.80; rays not visible on the cross-section without lens; inconspicuous on the radial; crystals in marginal ray cells scarce; intervacular pits minute, 0.003 mm. *Chickrassia*
2. Crystals in marginal ray cells absent; wood parenchyma chambered and containing numerous crystals in long chains; fibers with bordered pits.
- a. Vessels with spirals; wood light brown to red. *Azadirachta*
- b. Vessels without spirals.
- a¹. Fibers septate; rays uniseriate; wood light red. *Cipadessa*
- b¹. Fibers non-septate; rays multiseriate in part.
- a². Rays homogeneous; intervacular pits large, 0.0065 mm. diam.; wood light yellowish-brown; gum ducts absent. *Ekebergia*
- b². Rays heterogeneous; intervacular pits minute, 0.003 mm. diam.; wood greyish-pink or dark red; gum ducts present or absent.
- a³. Wood soft, greyish-pink; fibers thin-walled, free of contents; rays 1-3 cells wide. *Sandoricum*
- b³. Wood hard, dark red; fibers thick-walled, lumina filled with red gum; rays 1-4 cells wide. *Owenia*
- B. Terminal parenchyma absent; metatracheal in continuous or broken tangential lines 3 to 9 per mm.
- a. Rays homogeneous.
- a¹. Fibers non-septate; rays 1-4 cells wide; color variegated, light to dark reddish-brown with purple; wood parenchyma conspicuous. *Walsura*
- b¹. Fibers septate; rays 1-2 cells wide.
- a². Wood light yellowish-brown with golden luster; odor aromatic; hard, sp. gr. 0.82-0.98; concentric lines of wood parenchyma with crystals; fibers very thick-walled; mucilaginous

⁴ *Pseudocedrela* has aromatic odor when fresh.

- layers common *Reinwardtiidendron*
- b². Wood light to dark red without golden luster;
aromatic odor present or absent; soft to
moderately hard, sp. gr. 0.47–0.75; concentric
lines of wood parenchyma with crystals absent;
fibers thin to thick-walled; mucilaginous layers
absent *Guarea*
- b. Rays heterogeneous.
- a¹. Fibers non-septate.
- a². Rays uniseriate (1–2 cells wide in *T. emetica*
and *T. lanata*); wood light reddish-brown or
orange-brown *Trichilia*
- b². Rays 1–4 cells wide, mostly triseriate; wood
light to dark grey *Heynea*
- b¹. Fibers septate.
- a². Wood parenchyma chambered with crystals
in long chains.
- a³. Wood dark red or dark reddish-brown.
- a⁴. Multiseriate rays exclusively biseriate.
- a⁵. Rays not visible without lens on
the cross-section; wood parenchyma
not conspicuous on any section;
intervascular pits minute, 0.003 mm.
diam.; wood hard with aromatic
odor, sp. gr. 0.76–1.10 *Aglaia*
- b⁵. Rays barely visible without lens
on cross-section; wood parenchyma
conspicuous; intervacular pits large,
0.006 mm. diam.; wood without
distinctive odor, sp. gr. 0.57–0.73 *Synoum*
- b⁴. Multiseriate rays up to 3 cells wide;
wood parenchyma conspicuous; rays
not visible on cross-section without lens . . . *Dysoxylum*
- b³. Wood light yellow, light yellowish-brown,
or light pink; wood parenchyma con-
spicuous.
- a⁴. Wood light yellowish-brown, hard,
sp. gr. 0.71–0.90 *Lansium*
- b⁴. Wood cream color or light pink,
moderately hard, sp. gr. 0.56–0.69 *Chisocheton*
- b². Wood parenchyma without crystals.
- a³. Multiseriate rays 1–2 cells wide, mostly
uniseriate; wood parenchyma not con-
spicuous, barely visible without lens; in-
tervascular pits large, 0.006 mm. diam.;
wood light to dark red *Aphanamixis*
- b³. Multiseriate rays 1–3 cells wide, mostly
biseriate; wood parenchyma conspicuous on
all sections; intervacular pits minute,
0.003 mm. diam.; wood very dark red or
chocolate *Cabralea*

CONCLUSIONS

The three important systems of classification concerning the Meliaceae are those of Harms (6), DeCandolle (5), and Bentham and Hooker (3.) Harms has divided the family into three sub-families as follows: (1) Cedreloideae with two tribes, Cedreleae and Ptaeroxyleae, (2) Swietenioideae, (3) Melioideae with six tribes, Carapeae, Turraeeae, Vavaeeae, Melieae, Azadirachteae, and Trichilieae. The systems of DeCandolle and Bentham and Hooker are somewhat similar, the family in each case being divided into four tribes, Melieae, Trichilieae, Swietenieae, and Cedreleae. DeCandolle, however, is the only one who includes *Carapa* with the Swietenieae.

Although the writer has followed somewhat all three classifications, he has tried to construct a key on the basis of gross morphological characters which will conform more closely to the key derived from the structure of the wood. Such conformity obtains in the keys presented, at least with respect to the larger groups, *i.e.*, the sub-families Swietenioideae, Melioideae, and Lovoinoideae.

The genera *Cedrela*, *Carapa*, and *Xylocarpus* have been transferred to the sub-family Swietenioideae using as primary morphological characters the number of ovules in each cell of the ovary, and also on the basis of wood structure.

The genus *Lovoa* has been set aside by itself in the sub-family Lovoinoideae on the basis of gross morphological and anatomical characters.

The genera *Chloroxylon*, *Flindersia*, and *Ptaeroxylon* have caused botanists considerable trouble. Bentham and Hooker and DeCandolle have placed *Chloroxylon* and *Flindersia* with the Meliaceae and *Ptaeroxylon* with the Sapindaceae. Engler and Prantl have included *Chloroxylon* and *Flindersia* with the Rutaceae and *Ptaeroxylon* with the Meliaceae.

According to Solereder (15, p. 174, 194), the Rutaceae are characterized in a definite manner by the presence of schizogenous secretory cavities in the ground-tissue of the branches and of the leaf, giving rise to transparent dots on the leaf surface. The Meliaceae are characterized by the occurrence of secretory cells in the leaf-tissue and in the pith and cortex of the axes. The secretory cells are absent from the leaf in the genera *Chloroxylon* and *Flindersia*, but are present in the cortical tissue of the axes. Moreover, these two genera are further characterized by the occurrence of secretory cavities in the tissue of the leaf and cortex, and for this reason Engler transferred them to the Rutaceae. Capsular fruits, winged seeds, and ovaries with from 4–8 ovules in each cell must have been deciding factors for placing the two genera with the Meliaceae. The presence of secretory cells in the leaves of *Ptaeroxylon* caused Radlkofer to transfer this genus to the Meliaceae. The writer, after examining the woods of the three genera in question, decided that they resemble more closely those of the Rutaceae and therefore has not included them in the Meliaceae.

The results of the present investigation show, as the family now stands, great variation in both physical and anatomical characters. In fact they vary to such an extent that the writer has been unable to find a set of characters which will distinguish Meliaceae, as a whole, from other dicotyledonous families. There is also great variation in gross morphological characters.

The Swietenioideae is the only sub-family in which the genera form a distinct homogeneous group in respect to anatomical and morphological characters and it is the writer's opinion that it should be raised to the rank of a family, to be known as the Swieteniaceae.

When terminal parenchyma is found in *Khaya*, the woods of *Swietenia*, *Khaya*, and *Pseudocedrela* are indistinguishable with the exception that the woods of *Khaya* and *Pseudocedrela* have broader rays and *Pseudocedrela* is aromatic when fresh.

The sub-family Melioideae has been divided into three sections, *A*, *B*, and *C*, on the basis of morphological characters. Although these groups conform more closely than any others to the groupings derived from anatomical characters, the writer has found it impossible to get perfect anatomical and morphological correlation between certain genera within the various sections. If the groups are perfectly homogeneous in regard to wood structure, the morphological characters are not, and vice-versa.

In the Melioideae, section *B*, sub-section *I*, the genera *Trichilia*, *Heynea*, *Aphanamixis*, *Amoora*, *Synoum*, *Guarea*, *Chisocheton*, *Dysoxylum*, and *Cabrlea*, are characterized by simple pinnate or trifoliolate leaves, coriaceous capsules, ovary more than one-celled; and *Turreanthus* by a fleshy capsule and ovary one-celled. The chief anatomical characters are: heterogeneous rays (homogeneous in *Guarea*); fibers with bordered pits; wood parenchyma with numerous crystals in long chains (crystals absent in *Aphanamixis* and *Cabrlea*). With the exception of *Amoora* and *Turreanthus* the wood parenchyma occurs metatracheal in continuous to broken tangential lines 3 to 9 per mm. In *Amoora* and *Turreanthus* the parenchyma is diffuse and scarce.

In the Melioideae, section *B*, sub-section *II*, the genera *Cipadessa*, *Walsura*, *Lansium*, *Sandoricum*, *Aglaia*, *Ekebergia*, *Owenia*, and *Azadirachta* are characterized by simple pinnate or trifoliolate leaves, fruit berry-like (seed-like in *Azadirachta* and drupaceous in *Owenia*). The anatomical characters of the wood are: heterogeneous rays (homogeneous in *Ekebergia* and *Walsura*); fibers with bordered pits; wood parenchyma with crystals in long chains. *Cipadessa*, *Sandoricum*, *Ekebergia*, *Owenia*, and *Azadirachta* are characterized by terminal parenchyma in concentric lines 2 to 10 mm. apart. *Walsura*, *Lansium*, and *Aglaia* by metatracheal wood parenchyma in closely spaced tangential lines 3 to 9 per mm.

The genera *Reinwardtiadendron*, *Turraea*, *Vavaea*, and *Quivisia* are the only members of the family with exclusively simple leaves. The last three

genera form a homogeneous group in respect to wood structure and are the most primitive. The wood parenchyma is very scarce and diffuse; the rays are decidedly heterogeneous, *Turraea* and *Quivisia* contain uniseriate tips several times the length of the multiseriate portion; the vessels are minute and the end walls of the segments are strongly oblique. *Reinwardtiidendron*, however, with the exception of simple leaves, is very close to *Lansium* from a morphological and anatomical standpoint.

There is some doubt in the minds of botanists as to whether *Toona* is a distinct genus. Most of the English botanists have included *Toona* with *Cedrela*. Harms retains *Toona* as a separate genus and makes the following distinction:

1. Seeds winged only at the bottom. Disk longer than ovary. Distribution, America *Cedrela*
2. Seeds winged above or winged above and below. Disk shorter or as long as the ovary. Distribution, Asia, Australia, and the Philippines. *Toona*

Since the structure of the wood of *Toona* is identical with that of *Cedrela*, the writer has included *Toona* with *Cedrela*.

There are several genera within the Melioideae which are rather confusing as to their true identity. Two species of *Aphanamixis*, i.e., *A. grandiflora* Blume and *A. Rohituka* Pierre have been placed in the synonymy of *Amoora*. It is interesting to note that these species together with *Aphanamixis cumingiana* Harms are easily separable from those of *Amoora* from the standpoint of wood structure. The principal anatomical differences are:

| <i>Aphanamixis</i> | <i>Amoora</i> |
|---|---|
| Wood parenchyma numerous in continuous tangential lines 4-6 per mm. | Wood parenchyma scarce, not visible with lens; occurs diffuse as scattered cells. |
| Rays 1-2 cells wide, mostly uniseriate. | Rays 1-3 cells wide, mostly 2-3 cells. |

Amoora cucullata Roxb. seems to be more of an *Aphanamixis* than an *Amoora*. The wood parenchyma occurs in short tangential lines between the pores, but never forms continuous tangential lines. The rays are also 1-2 cells wide, mostly uniseriate.

With the exception of *Azadirachta integrifolia* Merr., the species of *Azadirachta* are synonyms of *Melia*. The anatomical differences between the two genera are:

| <i>Azadirachta</i> | <i>Melia</i> |
|---|--|
| Woods diffuse porous. | Woods ring-porous. |
| Minute pores do not form wavy tangential bands. | Minute pores form wavy tangential bands. |
| Gum ducts not observed. | Gum ducts present or absent. |
| Rays 1-3 cells wide. | Rays 1-8 cells wide. |

Many botanists have united *Xylocarpus* with *Carapa*. Harms makes the following distinction:

1. Upland trees. Leaves with many leaflets. Panicles large with many flowers. Fruit with woody husk. Distribution, trop. America, trop. Africa *Carapa*
2. Lowland trees. Leaves with few leaflets. Panicles small, with few flowers. Fruit with cork-like husk. Distribution, Asia, Philippines *Xylocarpus*

Although the woods resemble each other very much in structure, there are, however, the following differences:

| <i>Carapa</i> | <i>Xylocarpus</i> |
|--|--|
| Color usually light reddish-brown. | Color usually dark red. |
| Pores distinct as small to large pinholes. | Pores barely visible without lens. |
| Rays distinct on all sections. | Rays barely visible on the cross-section; inconspicuous on the radial. |
| Ripple marks absent. | Ripple marks always distinct. |

The slight differences between the two woods in question are probably due to environmental conditions.

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THE WOOD STRUCTURE OF A "PISTOL-BUTTED" MOUNTAIN HEMLOCK

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INTRODUCTION

Trees growing on hillsides frequently become bent over while young, due to the weight of snow or to insecure rooting. Their natural response to gravity causes them to grow erect again and even to bend back past the



TEXT FIG. 1. "Pistol-butted" western hemlocks (*Tsuga heterophylla*) growing on hillside. Pistol-butt caused by snow banks. Photo by Prof. C. F. Hottes.

vertical. This produces a shape which the woodsmen call "pistol-butted." All species of trees seem to be subject to this type of deformation to a greater or a lesser degree. Western hemlock (*Tsuga heterophylla*, text fig. 1) and coast spruce (*Picea sitchensis*) are most frequently, and western yellow pine (*Pinus ponderosa*) almost never, pistol-butted. Usually the steeper the slope the greater the number of pistol-butted trees. The loggers

are particularly interested in this type of tree because of the difficulty of falling and of bucking it. In undercutting such a tree, on one side the chips will break out from under the axe as if the wood was frozen whereas on the other side the wood is tough and does not break out readily when chopped.



TEXT FIG. 2. Cross section of a "pistol-butted" Douglas fir (*Pseudotsuga mucronata*), showing eccentric growth, thickened rings, and compression wood.

On cutting off the butt log the buckler frequently encounters "timber bind," that is, even though the log is lying in the clear, the saw binds when the log is partially cut through. This is apparently due to the wood on the one side of the log being under compression and the wood on the other side being under tension. As the saw cut divides these two sides a slight bending of the log at the point of cutting binds the saw.

On cutting off a pistol-butted tree at a height of one or two feet the trunk is usually found to be wider in the downhill-uphill direction than elsewhere and it is also distinctly eccentric with widening rings of a darker colored,

more solid appearing wood on the downhill side (text fig. 2). This is the so called compression wood, "redwood," or "Rothholz" of coniferous trees. Compression wood shrinks greatly on drying, particularly in the longitudinal direction. Thus lumber which contains some compression wood tends to warp and twist and be of inferior quality. Most of the timber-bearing area of the West is in mountainous country where the trees growing on steep hillsides are usually pistol-butted or at least develop some compression wood.

This paper deals with the structure of the wood of such a pistol-butted mountain hemlock (*Tsuga mertensiana*) in comparison with a normal, erect-growing tree of the same species.

LITERATURE REVIEW

Eccentric radial growth in plants has been known from earliest times (31). It is most frequently and easily seen, and has been most thoroughly studied, in trees; in fact little is known of its occurrence in shrubs or herbs (2). Jaccard (18) says "tension fibers," which occur only in eccentrically grown organs, are found only in trees, and that shrubs do not have them. This eccentric growth occurs in the stems of leaning or horizontal trees, in trees which are constantly exposed to the prevailing winds of a given region, and in trees which have been bent out of the vertical, either experimentally or naturally. It also occurs in the more or less horizontal branches of most trees. There seem to be some trees, chiefly dicotyledons, which do not show this eccentricity (18, 31). It was early pointed out (26, 38) and has since been confirmed by many writers (20, 31) that in the dicotyledons the greatest radial growth occurs on the upper side of horizontal branches whereas the opposite is true in the conifers. The conifers have been much more thoroughly investigated than have the dicotyledons.

This eccentricity is usually, though not necessarily always (3, 4, 36), accompanied by the formation of the so-called compression wood (also known as rothholz, redwood, bois rouge, glassy wood, and tennar). Many of these names are not satisfactory. Compression, for example, may not be the causal agency in all cases (3); and "redwood" refers also to the wood from other trees (*Sequoia*) or to a peculiar condition of the wood of Himalayan spruce (13, 24) which has nothing to do with true compression wood. Cieslar (4) says redwood does not occur in spruce stems which are concentric. However Mork (29) maintains it sometimes does occur in concentric stems which have been bent from one side to the other in response to gravity. When this occurs the compression wood is in the form of a spiral from the inner part of the tree toward the bark.

The cause of the formation of compression wood growth has been the subject of observation and experimentation for many years. The literature covering this subject has been discussed by Grossenbacher (14), and by Ursprung (35). These causes may be listed as gravity, light, longitudinal

pressure and tension, radial pressure of the bark, moisture, and nutritional relationships.

Gravity acting through the weight of the tree or branch producing a longitudinal compression or tension on the cambium was one of the causes to be elaborated by the earlier workers. Hartig (15) first maintained that mechanical compression produced redwood, later (16) he modified this view to include gravity working indirectly as a stimulus. That is, both compression and the gravity stimulus produce compression wood. Other workers (4, 9, 26, 33, 34, 38) have held the same views or have indicated still other possible factors as playing a part of more or less importance, as point and method of origin from the axis, injury, slope, and even unknown causes.

More recently Ewart and Jones (8) have carried on experimental work tending to show that both gravity and longitudinal compression cause the formation of compression wood but that pressure may modify or suppress the action of gravity. Mork (29) says gravity acts as a stimulus to cause the cambium to divide and form cells which bend the tree up to the vertical position. White (37) maintains that it is a purely gravity response and is not influenced by pressure or tension though light may modify the thickness of the tracheid walls. Burns (3) says compression does not produce redwood but that it is a "morphogenic response to a gravitation stimulus." Priestley and Tong (31) show that gravity acting on the cambium increases cell division and though this may be modified by the "condition of strain in which a branch is placed" it is independent of this and other factors such as light. Verrall (36) cites one case tending to show that strong compression as well as gravity may produce compression wood.

Light acting through its differential heating effect or as a tropic stimulus has been given by Müller as the cause for the formation of compression wood (30).

Radial pressure of the bark is given by Detlefsen (7) as the cause for eccentric growth, though this fails to explain the presence of compression wood on the tension side of the stem as found by Burns (3) and Hartig (16).

Gabnay (11) maintained that the greater specific gravity of the elaborated food of dicotyledonous trees as contrasted with conifers is the cause for the different reactions of these two types of trees when displaced from the vertical.

More branches on one side than on the other side, hence better nutrition on the branched side, has been given as the cause of eccentric growth by Müller (30) and Mer (28). This seems disproven by the experiments and observations of Burns (3) and Behre (1), though Fritz and Averell (10) found rings present in redwood only on that side of the tree having branches.

In conifers the compression wood has a reddish brown color when fresh and occurs usually in the wide, more solid appearing annual rings (16, 29). It loses this color upon drying unless treated with vaseline or fat (16). It

regains its color upon soaking in water. In both coniferous and dicotyledonous trees the annual rings containing compression wood can be distinguished with difficulty because there is no marked differentiation between the spring and summer wood.

In cross section the tracheids of well developed compression wood (and there are all degrees of its development) are very distinctive, being thick-walled, rounded with large intercellular air spaces (3, 16, 18, 21, 29, 31, 36). The cells are often of varying size, large and small being intermixed. They are found in more or less regular radial rows but usually not in regular tangential rows. The large intercellular spaces make it difficult to cut thin cross sections of wood without the sections falling apart (15, 36). Compression wood has resin ducts with heavier epithelial cells, and also contains more parenchyma and rays. In ring-porous dicotyledons geotropic wood (compression wood) has wider vessels and more summer wood than normal wood whereas in diffuse-porous dicotyledons the wood on the upper and lower side of branches is of the same general type (9).

The length of the tracheids in compression wood has been found by many authors (12, 15, 27, 29, 32, 36) to be less than in tension wood and normal wood. The radial diameter is likewise less (36). Hartig (16) ascribes this to the fact that cell division occurs more rapidly in compression wood than in normal wood or tension wood, hence each cambium initial has less time in which to stretch before another cell division takes place. This results in greater growth (wider rings and more cells) but also results in shorter, smaller elements. This idea is supported by the work of Priestley and Tong (31) and Jaccard and Frey (21) who maintain that thickening and lignification of the cell wall proceed more rapidly in compression wood, hence less time is allowed for elongation of the tracheid.

Hartig (15, 16) describes and figures the structure of compression wood (rothholz) as well as tension wood (zugholz). He says the primary wall is strongly lignified, the secondary wall is greatly thickened and is made up of 20-40 spiral bands closely laid together. The tertiary wall is lacking. Jaccard and Frey (21) have found the structure of compression tracheids and fibers to be the same as reported by Hartig. They have determined by means of polarized light the slope of the rows of micellae and find them to be steeper in the tension wood element than in the compression wood element of conifers, because the former elongate more. In the fibers of *Populus nigra* the micellar rows in tension wood are parallel to the long axis of the fiber whereas in the compression wood they are distinctly spiral. This difference the authors attribute partly to mechanical effects (tension on the upper side of the branch) and partly to the effects of water conduction. The slope of the spirals formed by the rows of micellae is influenced by the degree of elongation of the elements. In very slowly growing wood (3-6 cells per ring) the spirals were much steeper than in rapidly growing wood (500 cells per ring) because the elements of the former elongate more. The recent

work of Clark (5) indicates the possibility of determining compression wood and the slope of its micellar rows by means of X-rays.

The compression wood elements are more highly lignified than are the tension and normal wood elements (2, 21, 31, 34) which are largely cellulose and hemicellulose. These results based on staining reactions have been substantiated by the more recent chemical analyses of Johnsen and Hovey (23), Dadswell and Hawley (6), and Mork (29). This difference in chemical composition has a distinct effect on the physical properties of the wood (33) and on its usefulness for pulp (29). Priestley and Tong (31) point out the fact that lignification of the cell wall is a process distinct from division and growth of the cambium initials and is differently affected by gravity.

Burns (3) found compression wood to contain less moisture (38 percent) than normal wood (51 percent). Verrall (36) reports the same.

The dry weight of compression wood varies widely. Some of it is heavier than oak and practically all of it is heavier than normal wood (29). Typical compression wood is much harder than normal wood. The carpenters in certain regions (16) call it "nail hard" because it is almost impossible to drive a nail into it. It is able to withstand a greater compression strain than normal wood (3, 12, 17, 34, 36). Burns (3) reports compression wood twice as resistant to a pulling strain as normal wood, but others (17, 34, 36) find it less resistant. Its short, thick-walled cells are better able to withstand a compression strain than are the longer cells of tension wood or normal wood, the former of which is particularly resistant to a pulling strain (33). All agree that compression wood is of a glassy, brashy character and when broken in tension the fracture tends to be short whereas in normal wood the fracture is a very splintery one. This is of great importance in the trades.

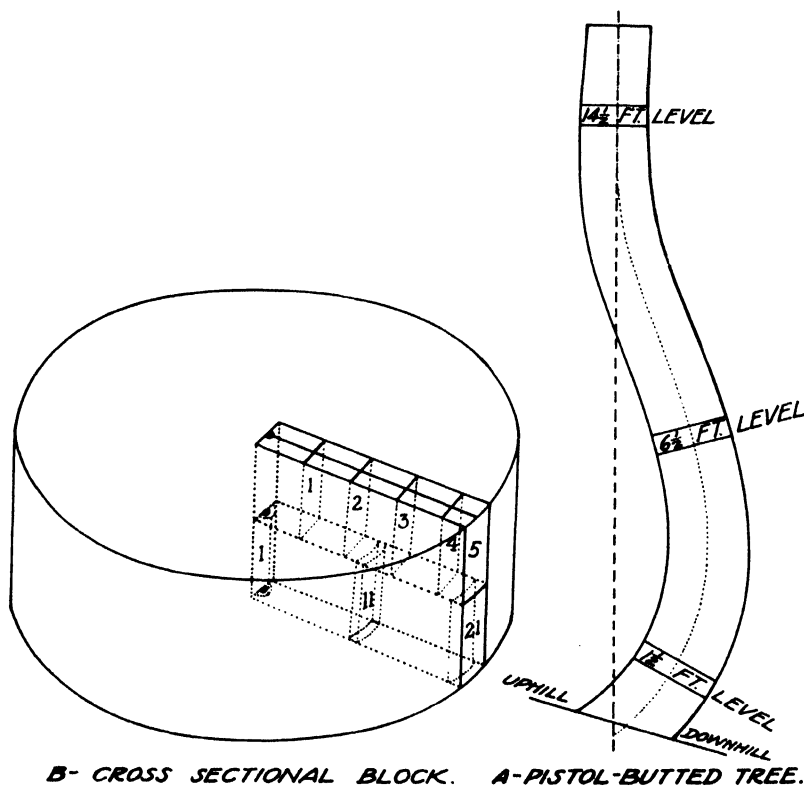
One of the most striking differences between compression wood and tension wood or normal wood is the excessive longitudinal shrinkage of compression wood. This amounts to as much as "2.46 percent as compared with 0.41 percent for normal wood" (25). Hartig (16) reports compression wood shrinking 1.28 percent and tension wood 0.09 percent in the longitudinal direction. Completely dried compression wood swells much more than tension wood in the longitudinal direction (3, 36). However, compression wood shrinks less volumetrically and in the tangential and radial directions than does normal or tension wood (16, 17, 36). Hartig explains such results on the basis that the intercellular spaces, so characteristic of compression wood, take up some of the shrinkage. The cause of greater longitudinal shrinkage and swelling is said by Koehler (25) to be due to the flatter slope of the micellar rows in the compression wood (35 degrees) as compared to that of normal wood (10 degrees).

This difference in shrinkage is the chief cause of warping in timbers containing some compression wood (3, 25, 29). Heck (17) describes a yellow pine beam, part of which was compression wood, which sagged when used and which developed cracks due to the greater longitudinal shrinkage

of the compression wood of the beam as contrasted with the normal wood of the beam. Hartig (16) says the peasants in certain wooded regions use dead spruce branches as weather indicators, because in wet weather they bend upward and in dry weather they bend downward due to the differential shrinking of the compression wood on the lower and the tension wood on the upper side of the branch.

MATERIALS AND METHODS

The wood used in this study was obtained from trees growing in a climax virgin timber forest at an elevation of about 3,500 feet near Goose Lake, on the Columbia National Forest in the Cascade Mountains of southwestern Washington. This forest consists of a dense, fairly young stand of western white pine (*Pinus monticola*), Douglas fir (*Pseudotsuga mucronata*), and white fir (*Abies nobilis* and *A. amabilis*) with scattering



B- CROSS SECTIONAL BLOCK. A-PISTOL-BUTTED TREE.

TEXT FIG. 3. A, Diagram of a pistol-butted mountain hemlock showing levels at which cross-sectional disks were cut. B, Diagram of a cross-sectional disk of the tree showing the radius along which wood was taken for study. Upper half of block divided into small pieces for cross-sectional measurements. Lower half used for tracheid measurements; rings 1, 11, 21, etc., used.

mountain hemlock (*Tsuga mertensiana*), western hemlock (*Tsuga heterophylla*), western red cedar (*Thuja plicata*) and lodgepole pine (*Pinus contorta*). A pistol-butted mountain hemlock growing on a moderate slope

was marked on its downhill side and then cut off at a height of $1\frac{1}{2}$ feet. A complete cross-sectional disk two inches thick was taken at this height. Similar disks were taken at heights of $6\frac{1}{2}$ feet, where the tree was bending back past the vertical, and at $14\frac{1}{2}$ feet where the tree was again straight (text fig. 3, A). Care was taken in all cases to mark the downhill and uphill radii on each disk.

A normal erect tree of mountain hemlock growing nearby on nearly level ground was cut down and cross-sectional disks taken at elevations of 2, 12, and 22 feet. On each of these disks four radii were measured, an average of these struck, and this average radius marked on the disk. The wood along this average radius was saved and used for further examination. The wood from this tree was used as a check in comparison with that from the pistol-butted specimen.

A narrow block of wood about half an inch wide was cut out of each disk along the desired radius. This block was divided into an upper and lower half. The upper half was used for cross-sectional measurements, the lower for tracheid length measurements (text fig. 3, B). The following radii were thus available for study:

Pistol-butted tree (No. 109)—

Downhill radius, $1\frac{1}{2}$ foot level
Uphill radius, $1\frac{1}{2}$ foot level
Sidehill radius, $1\frac{1}{2}$ foot level
Downhill radius, $6\frac{1}{2}$ foot level
Uphill radius, $6\frac{1}{2}$ foot level
Downhill radius, $14\frac{1}{2}$ foot level

Normal, erect (check) tree (No. 107)—Average radius, 2 foot level

Average radius, 12 foot level

Average radius, 22 foot level

For cross-sectional measurements the upper half-block was cut into pieces of convenient size for sectioning. These pieces were numbered consecutively from the pith to the bark. They were boiled to expel air and placed in glycerin-alcohol until ready for cutting. Cross-sections 30 microns thick were cut from these blocks on a sliding microtome, stained with safranin, and mounted in glycerin for study. The width of each ring was measured as well as the width of the spring and summer wood. From these measurements the percentage of summer wood in each ring was calculated. The average radial diameter of the tracheids was determined by counting the number of tracheids covered by a convenient number of spaces in the ocular micrometer and from this the average radial diameter of each tracheid calculated. Both spring and summer wood tracheids were measured in this way. In every case tracheids close to the dividing line between one year's growth and the next were measured, thus getting typical spring and summer wood tracheids and not transitional forms.

The average tangential diameter of the tracheids was obtained by calculation from a count of the number of tracheids covered by 50 ocular

spaces (530 microns). No consistent difference in tangential diameter was found between spring and summer wood tracheids, hence only those from the summer wood were measured. In a similar fashion the number of medullary rays occurring in 100 ocular spaces (1060 microns) was counted and their frequency of occurrence expressed in terms of the average distance in microns between adjacent rays. The degree of differentiation between spring and summer wood and the amount and degree of development of compression wood was estimated for each ring. Thickness of cell wall was measured under great magnification, in both spring and summer wood tracheids in occasional rings.

All of the cross-sectional data were averaged by groups of 10 rings each, from the pith to the bark, and are presented in that form in tables 1-4 and in all of the graphs.

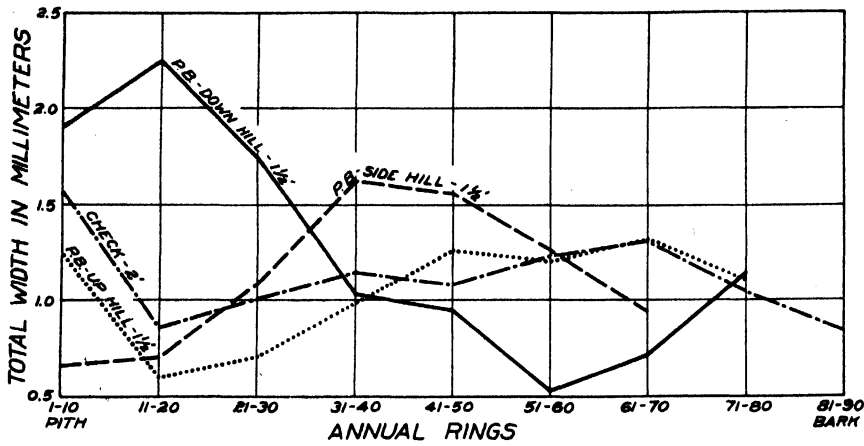
For tracheid length measurements the lower half-block (text fig. 3, *B*) was smoothed off and every tenth ring along the radius from the pith to the bark was marked. Thus rings 1, 11, 21, etc. from the pith outward to the bark were examined. In order that a random mount of the macerated material might contain tracheids from the spring wood and from the summer wood in exactly the same proportion in which they exist in the original ring, care was taken to use all the wood of a single ring, or if narrow two entire rings were used. Never were only parts of one or more rings taken. This was done in order to avoid any error due to possible differences in the length of spring tracheids and summer tracheids. The wood of the ring being examined was carefully cut out with a knife and these toothpick-like sticks were soaked overnight in water or in water with a few drops of nitric acid added.

When ready for examination the slivers of wood were gently boiled in half strength nitric acid until upon vigorous shaking they began to separate. Then a considerable amount of potassium chlorate was added and allowed to act for only a short time. The whole was then decanted into a beaker of water which stopped the action. This was very thoroughly mixed and a random sample of the tracheids removed with a pipette, separated on a slide and mounted in water. Only unbroken, perfect-ended tracheids were measured, a calibrated ocular micrometer and a low power objective being used. Each division of the ocular was equal to 21.7 microns. One hundred tracheids were measured from each ring examined and their average calculated. This number necessitated making several mounts before each of which the macerated material was well mixed. This resulted in a uniform sampling of the ring under investigation. Care was taken not to measure the same tracheid twice. No selection was exercised beyond avoiding broken and imperfect tracheids.

RESULTS

Total Width of Growth Rings

The total width of the annual rings varies widely from year to year along all of the radii but when averaged by 10-year periods or when the



TEXT FIG. 4. Average total width of the annual rings from the pith to the bark, expressed in millimeters. Pistol-butted (P.B.) tree at the 1½ foot level along the downhill, uphill, and sidehill radii. Check tree at the 2 foot level along the average radius.

graph representing the data is smoothed, a certain uniformity of trend is easily discernable (table I and text fig. 4). At the 1½ foot level the rings

TABLE I. Total Width of Growth Rings in Millimeters

| Ring Number Pith to Bark | Pistol-Butted Tree | | | | | | Check Tree | | |
|-----------------------------|--------------------|-------------|---------------|---------------|-------------|-------------------------------|-----------------|------------------|------------------|
| | 1½ Foot Level | | | 6½ Foot Level | | 14½ Foot Level Downhill | 2 Foot Level | 12 Foot Level | 22 Foot Level |
| | Down- hill | Up- hill | Side- hill | Down- hill | Up- hill | | | | |
| | | | | | | | | | |
| 1-10. | 1.90 | 1.24 | 0.66 | 1.78 | 1.30 | 2.00 | 1.56 | 1.60 | 1.73 |
| 11-20. | 2.25 | 0.60 | 0.71 | 2.28 | 1.33 | 1.57 | 0.87 | 1.26 | 1.69 |
| 21-30. | 1.74 | 0.70 | 1.09 | 1.64 | 0.69 | 1.56 | 1.01 | 1.07 | 1.50 |
| 31-40. | 1.03 | 0.99 | 1.63 | 1.31 | 1.17 | 1.44 | 1.14 | 1.03 | 1.41 |
| 41-50. | 0.95 | 1.26 | 1.56 | 1.15 | 1.66 | 1.31 | 1.08 | 1.28 | 1.19 |
| 51-60. | 0.53 | 1.20 | 1.26 | 0.84 | | 1.34 | 1.23 | 1.14 | 1.00 |
| 61-70. | 0.70 | 1.33 | 0.95 | 0.70 | | | 1.30 | 1.01 | |
| 71-80. | 1.14 | 1.10 | | | | | 1.06 | | |
| 81-90. | | | | | | | 0.83 | | |

along the downhill radius are unusually wide near the pith (2.07 mm.), but become comparatively narrow nearer the bark (0.92 mm.). The rings along the uphill radius, on the other hand, take just the opposite course (0.92 mm., and 1.21 mm.), while the rings along the sidehill radius are narrow near the pith (0.66 mm.) and bark (0.95 mm.) and wide midway between (1.63 mm.).

The rings of the check tree are intermediate in width (1.12 mm.) between those along the uphill and sidehill radii. The stumps of a considerable number of pistol-butted trees of various species in this same locality have been observed and the widening of the rings on the downhill side is common to all of them.

The unusual thickness of the rings along the downhill radius near the pith is undoubtedly a direct response to the greater stress on the downhill side and to the gravity stimulus in the leaning young tree. When this stress was taken care of by increased growth on the downhill side the rings became of essentially similar width along all three radii, downhill, uphill, and sidehill. At the 6½ foot level the relations are similar to those at the 1½ foot level. The check tree shows a slight increase in ring width from bark to pith and from the 2 foot level to the 22 foot level. This is characteristic of many other coniferous trees of various species growing in different habitats in this same locality.

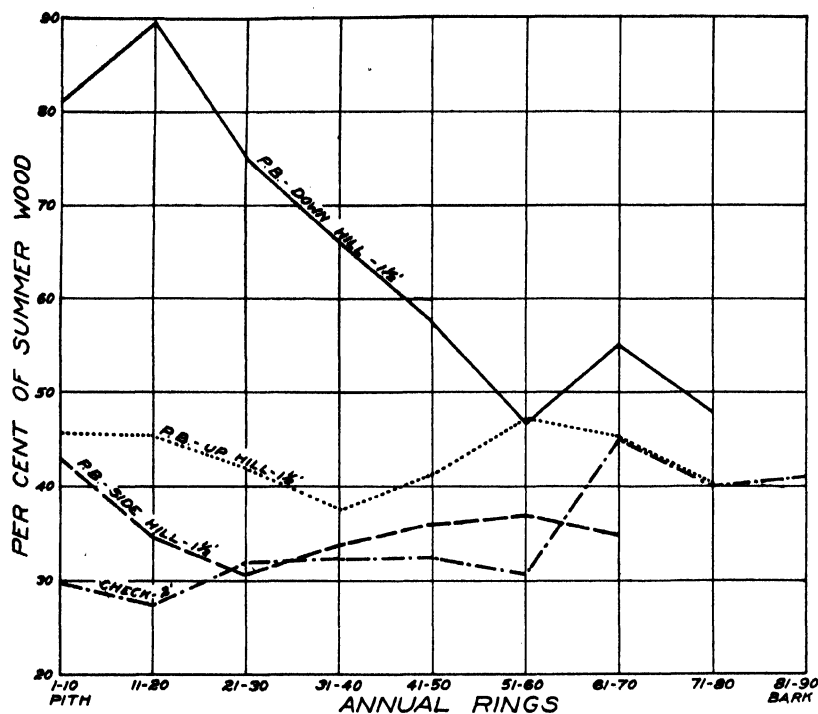
Percent of Summer Wood

The amount of spring and summer wood in every ring was measured and from this the percent of the ring occupied by summer wood was calculated. The compression wood where it occurred was, because of its thick walls, measured as summer wood. This seemed more desirable than measuring compression wood separately because the transition between spring wood and compression wood and between compression wood and summer wood was usually so gradual that the dividing line was impossible to determine accurately. Likewise all degrees of differentiation were found from well developed compression wood to normal spring wood or normal summer wood.

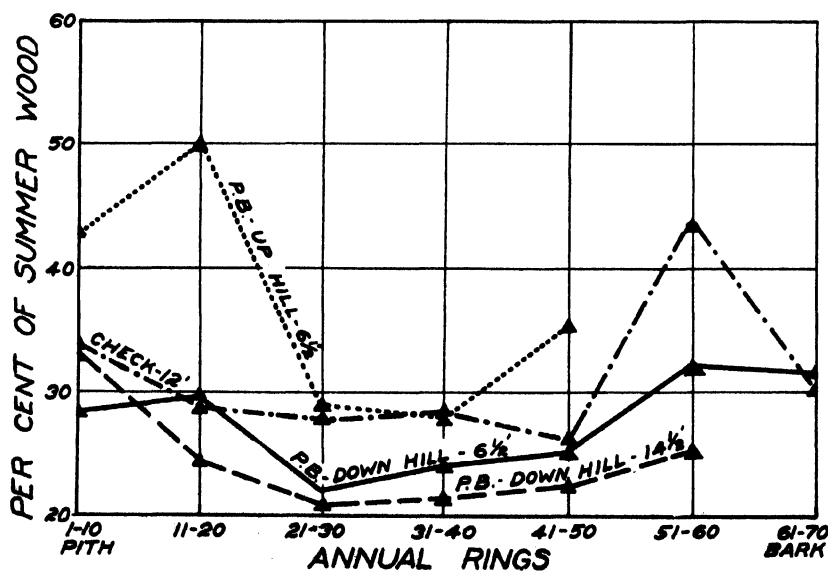
TABLE 2. *Percent of Summer Wood*

| Ring Number Pith to Bark | Pistol-Butted Tree | | | | | | Check Tree | | |
|-----------------------------|--------------------|-------------|---------------|---------------|-------------|-------------------------------|-----------------|------------------|------------------|
| | 1½ Foot Level | | | 6½ Foot Level | | 14½ Foot Level Downhill | 2 Foot Level | 12 Foot Level | 22 Foot Level |
| | Down- hill | Up- hill | Side- hill | Down- hill | Up- hill | | | | |
| 1-10..... | 81.0 | 45.7 | 42.9 | 28.4 | 42.6 | 33.1 | 29.7 | 33.7 | 27.1 |
| 11-20..... | 88.7 | 45.4 | 34.6 | 29.4 | 50.0 | 24.5 | 27.5 | 28.6 | 27.9 |
| 21-30..... | 75.3 | 42.1 | 30.7 | 21.8 | 29.0 | 21.1 | 32.1 | 27.7 | 32.3 |
| 31-40..... | 66.1 | 37.6 | 33.8 | 23.9 | 27.8 | 21.4 | 32.3 | 28.1 | 26.0 |
| 41-50..... | 57.6 | 41.3 | 35.7 | 25.1 | 35.2 | 22.5 | 32.5 | 26.1 | 29.5 |
| 51-60..... | 46.4 | 47.0 | 36.2 | 32.1 | | 25.7 | 30.7 | 44.0 | 34.6 |
| 61-70..... | 54.9 | 45.6 | 34.6 | 31.6 | | | 45.7 | 30.2 | |
| 71-80..... | 47.9 | 40.6 | | | | | 40.2 | | |
| 81-90..... | | | | | | | 40.9 | | |
| Average... | 64.7 | 43.2 | 35.4 | 27.5 | 36.9 | 24.7 | 34.5 | 31.2 | 29.6 |

Data given in table 2 and shown graphically in text figure 5 indicate the greater percent of summer wood along the downhill radius (64.7) especially



TEXT FIG. 5. Average percent of summer wood per annual ring from the pith to the bark. Pistol-butted (*P.B.*) tree at the $1\frac{1}{2}$ foot level along the downhill, uphill, and sidehill radii. Check tree at the 2 foot level along the average radius.



TEXT FIG. 6. Average percent of summer wood per annual ring from the pith to the bark. Pistol-butted (*P.B.*) tree at the $6\frac{1}{2}$ foot level, downhill and uphill radii; and $14\frac{1}{2}$ foot level, downhill radius. Check tree at the 12 foot level.

near the pith (84.9). The lesser amount of summer wood along the uphill (43.2 percent) and side hill (35.4 percent) radii is clearly shown. The summer wood along the sidehill radius is similar to that of the check tree at the 2 foot level (34.5 percent). At the $6\frac{1}{2}$ foot level (text fig. 6) the order is reversed and the uphill exhibits a greater amount of summer wood (36.9 percent) than does the downhill radius (27.5 percent). Text figure 3 *A* indicates that the $6\frac{1}{2}$ foot section was taken at a point where the tree was beginning to bend back past the vertical, thus being in effect a second pistol-butt with a curve similar to the one at the $1\frac{1}{2}$ foot level except reversed. This probably accounts for the reversal in percent of summer wood as well as differences in the size of tracheids, which will be discussed later. In the case of the check tree the percents of summer wood along the average radii at the 2, 12 and 22 foot levels are: 34.5, 31.2 and 29.6.

Compression Wood

The compression wood of mountain hemlock is similar to that described by Burns (3), Hartig (15), and others, that is, in its extreme development the cells are large, rounded, and thick walled with abundant intercellular air spaces. The cells are not in as definite rows as in normal wood. It may be added that there are all degrees of development of compression wood from that which can hardly be distinguished from spring or summer wood to that which is fully developed, as described above. Compression wood is usually found in the spring wood portion of the ring near the summer wood and may vary from a narrow band with spring wood on either side to a very broad band occupying the entire ring. Not only does compression wood vary in its degree of development and in its amount but a transitional stage between rings having compression wood and those having normal wood is a condition in which there is very poor differentiation between spring wood and summer wood, the spring cells being small and having walls almost as thick as the summer wood cells.

Observations were made on the amount of differentiation and the presence or absence and degree of compression wood formed in each ring.

At the $1\frac{1}{2}$ foot level along the downhill radius the first 5 rings next to the pith showed very little differentiation into spring and summer wood. Most of the spring wood cells were thick walled, making them look like summer wood cells.

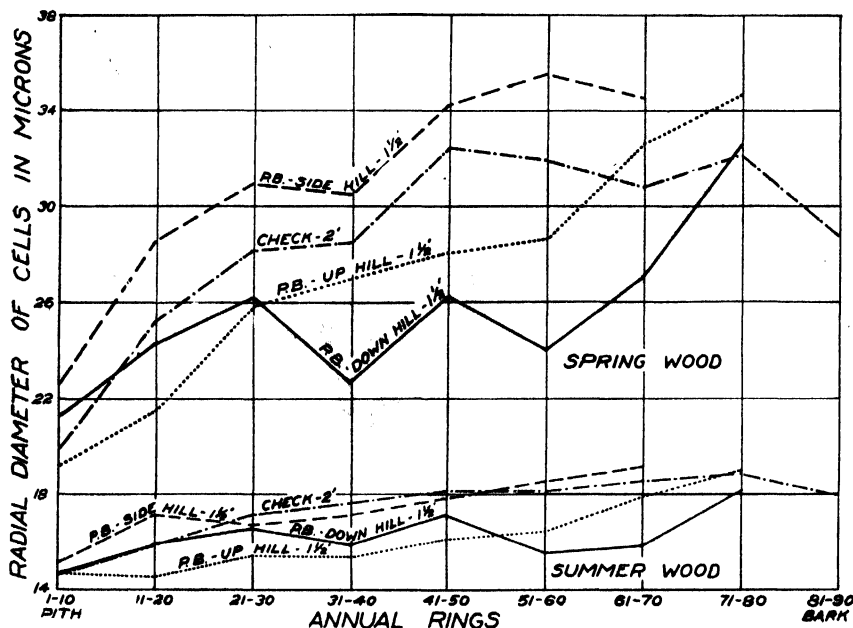
Rings 6-15 showed a slight development of compression wood but chiefly merely heavy walled cells. Rings 16-38 showed very well developed compression wood, with rounded cells and intercellular spaces. Rings 39-56 showed well developed compression wood. Rings 57-74 showed slightly developed compression wood. Rings 75-82 showed no indications of compression wood. Along the uphill and sidehill radii there was almost no compression wood visible anywhere, and most of the rings were well differentiated.

At the 6½ foot level along the downhill radius the 8, 11, and 19 rings from the pith showed compression wood whereas along the uphill radius the 2, 3, 13, 19, and 42-44 rings showed compression wood, and 28 other rings showed gradual differentiation from spring to summer wood. These poorly differentiated rings were always on either side of and near the compression wood, being apparently a transition from normal wood to compression wood. At the 14½ foot level a very few scattered rings showed compression wood.

In the check tree a few scattered rings at each of the levels (2, 12, and 22 feet) showed compression wood or gradual differentiation from spring to summer wood.

Radial Diameter of the Tracheids

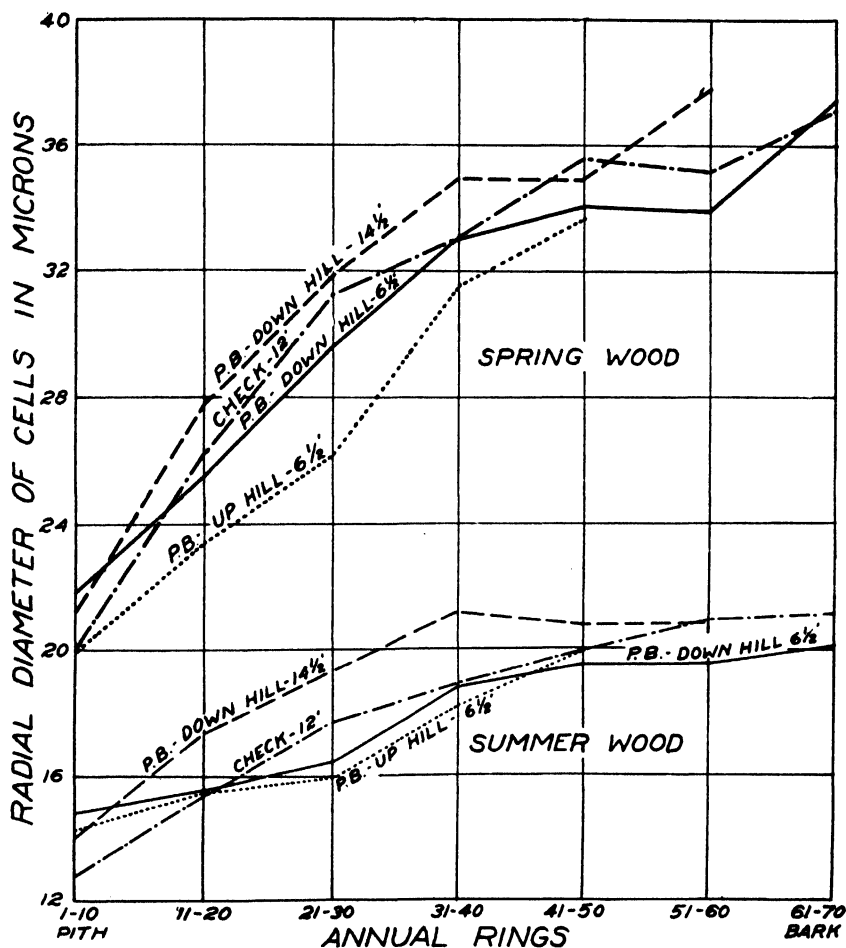
The average radial diameter of the tracheids of both spring and summer wood increases from the pith toward the bark in all cases (text figs. 7 and 8).



TEXT FIG. 7. Average radial diameter of the tracheids of spring and summer wood from pith to bark, expressed in microns. Pistol-butted (P.B.) tree at the 1½ foot level along the downhill, uphill and sidehill radii. Check tree at the 2 foot level along the average radius.

This increase is greater in the case of spring wood tracheids than in the case of summer wood tracheids. The radial diameter of the tracheids of both spring and summer wood at the 1½ foot level is smaller along the downhill radius (spring, 25.5 microns; summer, 16.2 microns), larger along the uphill radius (spring, 27.1 microns; summer, 16.0 microns), and largest along the sidehill radius (spring, 30.9 microns; summer, 17.4 microns), an increase of 5.4 microns (21.2 percent) in the case of the spring wood tracheids and 2.2

microns (13.7 percent) in the case of summer wood tracheids. The size of the tracheids from the check tree at the 2 foot level (text fig. 7) is slightly smaller (spring, 28.6 microns; summer, 17.5 microns) than that of those along the sidehill radius (see tables 3 and 4).



TEXT FIG. 8. Average radial diameter of the tracheids of spring and summer wood from pith to bark, expressed in microns. Pistol-butt (P.B.) tree at the 6½ foot level along the downhill and uphill radii, and at the 14½ foot level along the downhill radius. Check tree at the 12 foot level along the average radius.

In contrast with the above, the tracheids at the 6½ foot level show (text fig. 8) a reversal in size in that the radial diameter of the tracheids of both spring and summer wood is greater along the downhill radius (spring, 30.7 microns; summer, 18.4 microns), than along the uphill radius (spring, 26.9 microns; summer, 16.8 microns) a difference of 3.8 microns (14.1 percent) for the spring wood and 1.6 microns (9.5 percent) for the summer wood tracheids. The tracheids of the check tree at the 12 foot level are slightly larger

(spring, 31.2 microns; summer, 18.1 microns) than those of the pistol-butted tree along the downhill radius at the 14½ foot level (spring, 31.4 microns; summer, 18.9 microns). This seems to indicate that the tracheids along the downhill radius at the 14½ foot level are normal in size and show no differences due to compression or tension. This is to be expected as the 14½ foot section was cut from the erect portion of the tree (text fig. 3 A).

TABLE 3. *Radial Diameter of the Tracheids of Spring Wood in Microns*

| Ring Number Pith to Bark | Pistol-Butted Tree | | | | | | Check Tree | | |
|-----------------------------|--------------------|-------------|---------------|---------------|-------------|-------------------------------|-----------------|------------------|------------------|
| | 1½ Foot Level | | | 6½ Foot Level | | 14½ Foot Level Downhill | 2 Foot Level | 12 Foot Level | 22 Foot Level |
| | Down- hill | Up- hill | Side- hill | Down- hill | Up- hill | | | | |
| I-10. | 21.3 | 19.1 | 22.6 | 21.7 | 19.9 | 21.3 | 19.9 | 20.1 | 22.6 |
| 11-20. | 24.3 | 21.5 | 28.4 | 25.4 | 23.4 | 27.8 | 25.3 | 26.3 | 28.3 |
| 21-30. | 26.2 | 25.7 | 30.9 | 29.7 | 26.1 | 31.8 | 28.2 | 31.3 | 32.0 |
| 31-40. | 22.6 | 26.9 | 30.5 | 32.9 | 31.6 | 34.9 | 28.4 | 33.0 | 36.8 |
| 41-50. | 26.3 | 28.0 | 34.3 | 34.0 | 33.6 | 34.9 | 32.4 | 35.5 | 37.2 |
| 51-60. | 24.0 | 28.6 | 35.1 | 33.8 | | 37.7 | 31.8 | 35.3 | 33.4 |
| 61-70. | 27.0 | 32.6 | 34.5 | 37.4 | | | 30.7 | 37.1 | |
| 71-80. | 32.5 | 34.7 | | | | | 32.2 | | |
| 81-90. | | | | | | | 28.7 | | |
| Average... | 25.5 | 27.1 | 30.9 | 30.7 | 26.9 | 31.4 | 28.6 | 31.2 | 31.7 |

The radial diameter of the tracheids increases with increasing height in the tree. This is shown in the check tree by comparing the size of the tracheids at the 2 foot level (spring, 28.6 microns; summer, 17.5 microns) with that at the 12 foot level (spring, 31.2 microns; summer, 18.1 microns) and the 22 foot level (spring, 31.7 microns; summer, 18.5 microns). In the above case the increase is 3.1 microns (10.9 percent) for the spring wood

TABLE 4. *Radial Diameter of the Tracheids of Summer Wood in Microns*

| Ring Number Pith to Bark | Pistol-Butted Tree | | | | | | Check Tree | | |
|-----------------------------|--------------------|-------------|---------------|---------------|-------------|-------------------------------|-----------------|------------------|------------------|
| | 1½ Foot Level | | | 6½ Foot Level | | 14½ Foot Level Downhill | 2 Foot Level | 12 Foot Level | 22 Foot Level |
| | Down- hill | Up- hill | Side- hill | Down- hill | Up- hill | | | | |
| I-10. | 14.7 | 14.7 | 15.2 | 14.8 | 14.4 | 14.1 | 14.6 | 12.7 | 12.5 |
| 11-20. | 15.9 | 14.5 | 17.2 | 15.6 | 15.4 | 17.4 | 15.9 | 15.4 | 16.2 |
| 21-30. | 16.5 | 15.4 | 16.7 | 16.4 | 15.9 | 19.3 | 17.2 | 17.7 | 18.8 |
| 31-40. | 15.8 | 15.5 | 17.2 | 18.7 | 18.3 | 21.2 | 17.6 | 18.9 | 21.2 |
| 41-50. | 17.2 | 16.1 | 17.7 | 19.5 | 19.9 | 20.7 | 18.2 | 19.9 | 20.7 |
| 51-60. | 15.6 | 16.5 | 18.6 | 19.5 | | 20.8 | 18.2 | 20.9 | 21.3 |
| 61-70. | 15.8 | 17.9 | 19.1 | 20.1 | | | 18.6 | 21.0 | |
| 71-80. | 18.2 | 19.0 | | | | | 18.8 | | |
| 81-90. | | | | | | | 18.0 | | |
| Average... | 16.2 | 16.0 | 17.4 | 18.4 | 16.8 | 18.9 | 17.5 | 18.1 | 18.5 |

tracheids and 1.0 microns (6.0 percent) for the summer wood tracheids. This increase is also noticeable in the case of the tracheids along the downhill radii in the pistol-butted tree at the various levels as discussed above. The increase from the $1\frac{1}{2}$ foot level to the $14\frac{1}{2}$ foot level is 5.9 microns (23.1 percent) in the case of the spring wood tracheids and 2.8 microns (16.7 percent) in the case of the summer wood tracheids.

In every case discussed above, the increase in the radial dimension of the tracheids whether along different radii (downhill, uphill) at the same level or whether along the same radius at different levels (2, 12, 22, etc., feet) is greater in the case of the spring wood tracheids (average 17.3 percent) than in the case of the summer wood tracheids (average 11.5 percent). This would seem to indicate that the spring wood tracheid initials are more sensitive to those stimuli which act on the undifferentiated cells of the cambium or on the xylem mother cells than are the summer wood tracheid initials. Just what the stimuli are is not known but from a survey of the work which has been done by others along this line, they are probably compression, tension, the morphogenic stimulus of gravity, the accumulation of food in the lower part of the curved base of the tree, and changing food supply due to the changing relation between crown size as the tree becomes older and the increased number of growing cells.

Tangential Diameter of the Tracheids

The average tangential diameter of the tracheids of the summer wood increases from the pith to the bark and from the base of the tree toward the top in a manner similar to but not as pronounced as the increase in radial diameter. In the main the order of size is similar to that of the radial dimension though not so definite and free from exception. The tracheids along the downhill radius at the $1\frac{1}{2}$ foot level are larger near the pith and only later equal and are exceeded by those along the uphill radius. However, those along the sidehill radius are distinctly larger than either. This last is

TABLE 5. *Tangential Diameter of the Tracheids of Summer Wood in Microns*

| Ring Number Pith to Bark | Pistol-Butted Tree | | | | | | Check Tree | | |
|-----------------------------|---------------------------|---------|-----------|---------------------------|---------|--|--------------|---------------|---------------|
| | $1\frac{1}{2}$ Foot Level | | | $6\frac{1}{2}$ Foot Level | | $14\frac{1}{2}$ Foot Level Downhill | 2 Foot Level | 12 Foot Level | 22 Foot Level |
| | Down-hill | Up-hill | Side-hill | Down-hill | Up-hill | | | | |
| 1-10. | 19.3 | 18.7 | 20.5 | 19.8 | 17.8 | 18.5 | 19.8 | 17.3 | 18.2 |
| 11-20. | 20.9 | 19.5 | 24.2 | 20.4 | 20.9 | 21.2 | 22.6 | 20.7 | 21.8 |
| 21-30. | 22.0 | 21.2 | 24.2 | 22.6 | 22.5 | 25.7 | 23.0 | 24.4 | 25.5 |
| 31-40. | 23.8 | 22.6 | 26.2 | 24.0 | 22.9 | 27.0 | 24.2 | 26.8 | 26.7 |
| 41-50. | 23.1 | 23.0 | 27.2 | 26.5 | 27.5 | 26.9 | 26.2 | 27.5 | 29.1 |
| 51-60. | 23.7 | 23.7 | 28.9 | 28.2 | | 28.5 | 25.9 | 27.9 | 30.5 |
| 61-70. | 25.9 | 25.9 | 27.9 | 27.3 | | | 28.3 | 27.9 | |
| 71-80. | 25.7 | 26.8 | | | | | 28.2 | | |
| 81-90. | | | | | | | 28.6 | | |
| Average. . . | 23.1 | 22.7 | 25.6 | 24.1 | 22.3 | 24.6 | 25.2 | 24.6 | 20.3 |

in accord with the radial dimension. The average dimension of the tracheids along these radii is: downhill, 23.1 microns; uphill, 22.7 microns; and sidehill, 25.6 microns. The check tree at the 2 foot level is intermediate, 25.2 microns. The 6½ foot and 14½ foot levels show trends similar to that at the 1½ foot level (table 5). It appears that the tangential dimension of the tracheid initials is not effected by the several stimuli to the same extent as is the radial dimension discussed above.

Thickness of Tracheid Walls

With the exception of the rings near the pith the thickness of the wall of either spring wood tracheids or summer wood tracheids does not vary consistently with the position of the ring on either the horizontal or the vertical axis. The rings near the pith are poorly or not at all differentiated into spring and summer wood. The tracheids in rings near the pith are smaller in both the radial and tangential diameters and the spring wood tracheids in such rings have much thicker walls (6.4 microns) than the spring wood tracheids farther away from the pith (4.9 microns). The summer wood tracheids near the pith have walls of about the same thickness or even slightly thinner than those farther away from the pith. This condition is most pronounced in the first and second rings from the pith but gradually changes to the normal condition within from 5-10 rings of the pith.

There is no significant difference in the thickness of the walls of the spring and summer wood tracheids on the downhill, uphill, or sidehill radii of the pistol-butted tree nor in comparison with the check tree. The average of all measurements from all levels of both trees gives the following result; spring wood tracheids, 5.2 microns; summer wood tracheids, 11.1 microns. The various stimuli acting on the tracheids do not effect the thickness of the cell wall but seem rather to bring about the production of more summer wood tracheids or more compression wood tracheids of a smaller size.

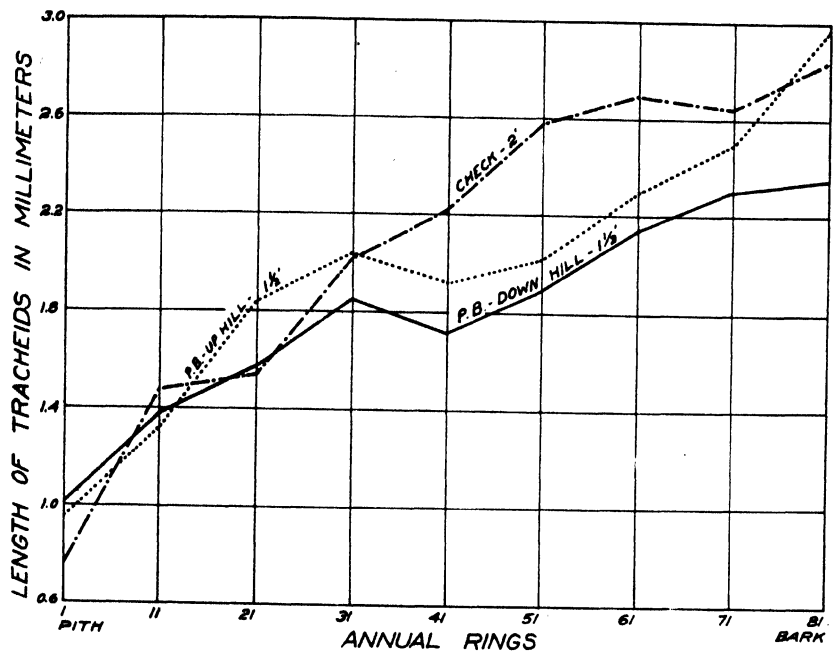
Frequency of Occurrence of the Medullary Rays

No marked correlation exists between the number of medullary rays per given unit of tangent and the position of the ring on the horizontal or the vertical axis of the tree. The number of rays in a given unit of tangent varies, being slightly more numerous along the downhill radii at the 1½ foot level and the 6½ foot level than along the uphill radii with the sidehill radius intermediate. Averaging all radii at all levels in both trees we find a ray every 183 microns. In all cases a slightly greater number of rays is noticeable near the pith. This is particularly well developed in the check tree. At the 2 foot level there is a ray every 163 microns in the first twenty rings from the pith and every 200 microns in the 70th to the 90th rings.

Length of Tracheids

The lengths of 5,600 tracheids are averaged and summarized in table 6. The average length of all tracheids measured is 2.11 mm. with the maximum

for an individual ring, 3.22 mm. for the 71st ring at the 12 foot level in the check tree, and the minimum .74 mm. for the first ring at the same level in the same tree. The longest single tracheid measured is 4.23 mm. long, the



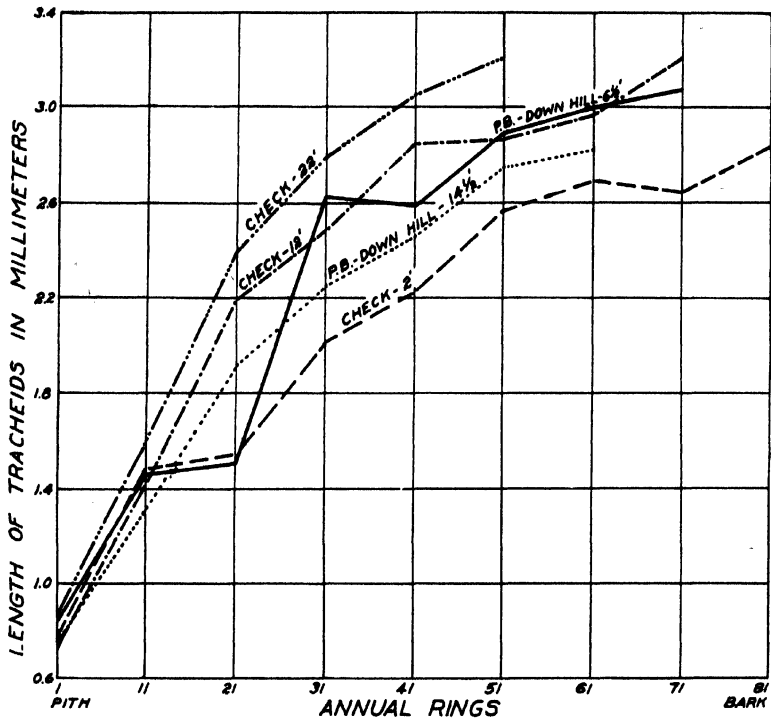
TEXT FIG. 9. Average length of the tracheids from every tenth ring from the pith to the bark, expressed in millimeters. Each point represents the average of 100 tracheids. Pistol-butted (P.B.) tree at the 1½ foot level along the downhill and uphill radii. Check tree at the 2 foot level along the average radius.

shortest is 0.37 mm. long. The tracheids vary considerably in length in any given ring but the measuring of 100 tracheids in each ring studied brings out clearly the differences in average length.

TABLE 6. Length of Tracheids in Millimeters

| Ring Number Pith to Bark | Pistol-Butted Tree | | | | Check Tree | | |
|-----------------------------|--------------------|--------|---------------------------|----------------------------|--------------|---------------|---------------|
| | 1½ Foot Level | | 6½ Foot Level Downhill | 14½ Foot Level Downhill | 2 Foot Level | 12 Foot Level | 22 Foot Level |
| | Downhill | Uphill | | | | | |
| I..... | 1.01 | 0.96 | 0.84 | 0.75 | 0.77 | 0.74 | 0.85 |
| 11..... | 1.38 | 1.33 | 1.45 | 1.31 | 1.48 | 1.42 | 1.60 |
| 21..... | 1.58 | 1.84 | 1.50 | 1.93 | 1.54 | 2.20 | 2.39 |
| 31..... | 1.86 | 2.04 | 2.62 | 2.26 | 2.01 | 2.49 | 2.80 |
| 41..... | 1.72 | 1.92 | 2.59 | 2.47 | 2.23 | 2.86 | 3.06 |
| 51..... | 1.90 | 2.03 | 2.90 | 2.75 | 2.58 | 2.88 | 3.21 |
| 61..... | 2.14 | 2.30 | 3.00 | 2.82 | 2.70 | 2.98 | |
| 71..... | 2.31 | 2.50 | 3.07 | | 2.66 | 3.22 | |
| 81..... | 2.36 | 2.97 | | | 2.85 | | |
| Average... | 1.81 | 1.99 | 2.25 | 2.04 | 2.09 | 2.35 | 2.32 |

In all cases the tracheids increase in length from the pith to the bark, from about .8 mm. to about 2.8 mm. At first this increase is rapid but later becomes less rapid with greater fluctuations (text figs. 9 and 10). This is particularly well shown in the case of the curve representing the check tree. The check tree also shows a characteristic feature of the wood structure of conifers generally, namely, the increase in length of the tracheids from the base toward the top of the tree (text fig. 10). At the pith the



TEXT FIG. 10. Average length of the tracheids from every tenth ring from the pith to the bark, expressed in millimeters. Each point represents the average of 100 tracheids. Pistol-butted (P.B.) tree at the 6½ and 14½ foot levels along the downhill radii. Check tree at the 2, 12, and 22 foot levels along the average radii.

tracheids are nearly all the same length but farther away from the pith the curves gradually diverge until, at the 51st ring where all three levels can be directly compared, the tracheids are 2.58 mm. long at the 2 foot level, 2.88 mm. long at the 12 foot level, and 3.21 mm. long at the 22 foot level. The curves representing the length of the tracheids from the pistol-butted tree are not as steep as those of the check tree. Likewise the increase in the length of the tracheids from the base toward the top of the tree is more rapid in the case of the check tree than in the case of the pistol-butted tree.

At the 1½ foot level of the pistol-butted tree, the tracheids average shorter along the downhill radius (1.81 mm.) than they do along the uphill

radius (1.99 mm.), but both are shorter than the tracheids along the average radius of the check tree at the 2 foot level (2.09 mm.) except near the pith (text fig. 9). The shorter tracheids at the $14\frac{1}{2}$ foot level as compared to the $6\frac{1}{2}$ foot level are very unusual and at present unexplainable. Out of about 16,000 measurements made on normal lodgepole pine the curves representing tracheid length have almost never crossed each other except at heights of 40 feet or more on the tree.

DISCUSSION

One of the most interesting features of this study is the reversal which takes place in the value of many of the criteria in passing from the $1\frac{1}{2}$ foot level to the $6\frac{1}{2}$ foot level. That is, the percent of summer wood is greater, the compression wood is better developed, and the radial diameter of both spring and summer wood tracheids is smaller along the downhill radius at the $1\frac{1}{2}$ foot level than along the uphill radius, whereas this condition is exactly reversed at the $6\frac{1}{2}$ foot level. It is likely that this reversal would also have been found to occur in the length of the tracheids had they been measured along the uphill radius at the $6\frac{1}{2}$ foot level. The criteria of total width of ring and tangential diameter of tracheids do not show this reversal, however.

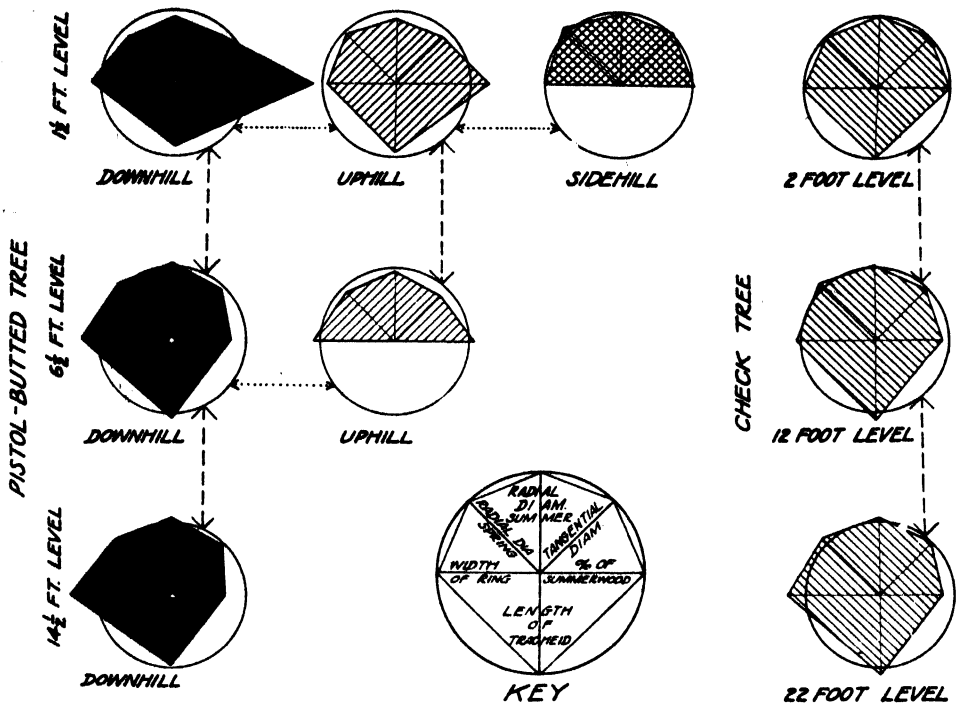
The reason for this reversal seems to lie in the fact that the leaning tree in responding to the stimulus of gravity grew erect and, as is usually the case in the gravity responses of stems, grew past the vertical. Therefore, its former upper side became the lower side and responded to the stimulus of gravity in the usual fashion. A similar instance is reported by Burns (3) in the case of a tree bent over by a wire sling. The part above the sling erected itself in response to gravity. A cross section showed compression wood on the lower side early in the season's growth and none later and compression wood on the upper side later in the season. Burns says of this, "The explanation of this phenomenon lies in the fact that when it erected itself in response to the stimulus of gravity it did not stop in a perpendicular position but went beyond so that during the later part of the growing season the conditions were reversed and the former upper side was later actually the lower side. In this position redwood was produced." Hartig (16) cites a similar case of redwood produced on the former upper side of a lateral branch which grew erect to replace a lost leader.

SUMMARY

All the data have been summarized in text figure 11, using a circular or radial graph¹ in which the six radii represent the different criteria as

¹ In a recently published article by Lutz in *Ecology* 11: 1-29, 1930, a graph similar to this one was used to characterize plant communities. Lutz used four criteria: (1) abundance, (2) frequency, (3) number of size classes, and (4) basal area per acre, and applied them only to the trees, in some cases only the dominant trees. He called his chart a "phyto-

indicated in the key figure. Rather than assign a definite value to each unit of the radius, all of the data have been expressed in proportion to the check tree at the 2 foot level, which is considered as unity (100), the inscribed figure exactly filling the circle.



TEXT FIG. 11. Summary of the averages of all measurements on the pistol-butted and the check tree. Each of the six criteria measured along a given radius (see key figure) and expressed in proportion to the measurements for the check tree at the 2 foot level considered as 100.

The size and shape of the six-sided figure enables one to judge the value of each of the criteria as well as the value of all of the criteria considered together. The length of the tracheids was not determined for the sidehill radius, $1\frac{1}{2}$ foot level and the uphill radius, $6\frac{1}{2}$ foot level. From the graph it can be determined that the criterion of radial diameter of the summer wood tracheids varies least from unity while the criterion of percent of summer graph." This name appears to me to be an unfortunate one as "phytograph" means "plant graph" and yet criteria 3 and 4 are applicable chiefly or wholly to trees and not to all kinds of plants. Since this type of graph is applicable to so many kinds of data using widely different criteria the name "phytograph" seems unnecessary. It is conceivable that this type of graph could be used to represent both plant criteria and insect depredations in which case the term "phytograph" could not be applied. What is needed is a general term to designate this type of graph similar to the terms "piegraph" and "bargraph" applied to other more familiar types.

wood varies most. The sidehill radius, $1\frac{1}{2}$ foot level, is most nearly like the check tree, 2 foot level. The width of ring increases with increasing height in the check tree while the percent of summer wood decreases. All of these facts and others mentioned in the discussion or in the summary can readily be determined from the summary graph.

1. The structure of the wood of a pistol-butted and a straight mountain hemlock (*Tsuga mertensiana*) growing at 3,500 feet elevation in the Cascade Mountains of southwestern Washington was examined.

2. The pistol-butted tree was examined at the $1\frac{1}{2}$, $6\frac{1}{2}$, and $14\frac{1}{2}$ foot levels along the downhill, uphill, or sidehill radii, and the straight tree at the 2, 12, and 22 foot levels along the average radius.

3. The total width of the rings was measured and averaged by groups of 10 rings each. At the $1\frac{1}{2}$ foot level along the downhill radius, the rings are very wide near the pith (2.07 mm.), but much narrower near the bark (0.92 mm.). The rings along the uphill radius average narrower, but are widest near the bark. Along the sidehill radius they are narrower near the pith and bark and wide midway between. The leaning of the pistol-butted tree seems to produce a greater width of the rings near the pith on the downhill side. This stimulus does not seem to be active in the last 10–20 years of the life of this tree as the rings near the bark are about equal along all radii.

4. The check tree at the 2 foot level has rings more or less intermediate in width from pith to bark (1.12 mm.).

5. At the $6\frac{1}{2}$ foot level the width of the rings is similar to that at the $1\frac{1}{2}$ foot level, though not as pronounced.

6. The proportion of the ring occupied by summer wood, expressed in percent, is as follows: $1\frac{1}{2}$ foot level, downhill 64.7 percent (very high near the pith, 84.9 percent); uphill 43.2 percent; sidehill 35.4 percent. Check tree, 2 foot level, 34.5 percent.

7. The percent of summer wood is exactly reversed at the $6\frac{1}{2}$ foot level as compared to the $1\frac{1}{2}$ foot level. It is greater along the uphill radius (36.9 percent) than along the downhill radius (27.5 percent).

8. Compression wood is well developed near the pith along the downhill radius at the $1\frac{1}{2}$ foot level. It gradually disappears toward the bark. There is practically none on the uphill or sidehill radii.

9. This is reversed at the $6\frac{1}{2}$ foot level, there being more compression wood along the uphill than along the downhill radius.

10. The radial diameter of the tracheids increases from the pith to the bark, the increase being greater in spring wood than in summer wood tracheids.

11. At the $1\frac{1}{2}$ foot level the radial diameter of the tracheids is least along the downhill radius, intermediate along the uphill radius, and greatest along the sidehill radius, with the check tree at the 2 foot level somewhat intermediate.

12. This is reversed at the $6\frac{1}{2}$ foot level, the radial diameter of the

tracheids being greatest along the downhill radius and least along the uphill radius.

13. Wherever a change in the radial diameter of the tracheids takes place, whether from pith to bark, from the 2 foot level to the 22 foot level, or from downhill radius to uphill radius at a given level, the change is always greater in the spring wood tracheids (17.3 percent) than in the summer wood tracheids (11.5 percent). The summer wood seems more conservative in its response to stimuli than is the spring wood.

14. The radial size of the tracheids increases also with increasing height in the tree.

15. The tangential diameter of the tracheids increases from pith to bark, from the base toward the top of the tree and along the different radii in a manner similar to the radial diameter, but not in as definite a fashion.

16. There is no consistent variation in thickness of cell wall that can be correlated with position on the horizontal or vertical axes of the tree. The average thickness is; spring wood, 5.2 microns; summer wood, 11.1 microns.

17. No very marked variation in the number of medullary rays per unit of tangent is noticeable. A medullary ray occurs every 183 microns.

18. The measurement of 5,600 tracheids in both trees gives an average length of 2.11 mm., an absolute maximum of 4.23 mm., and an absolute minimum of 0.37 mm.

19. The maximum length for a single ring is 3.22 mm. (71st ring at the 12 foot level, check tree) and the minimum 0.74 mm. (1st ring at same level, same tree).

20. The tracheids increase in length in all cases from the pith to the bark (0.8 mm. to 2.8 mm.), at first rapidly, then more slowly with greater fluctuations.

21. The tracheids also increase in length from the base toward the top of the tree.

22. The tracheids are shorter (1.81 mm.) along the downhill radius than along the uphill radius (1.99 mm.) both being shorter than those in the check tree at the 2 foot level (2.09 mm.).

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GAMETOPHYTE DEVELOPMENT IN *REBOULIA* *HEMISPHERICA*

SISTER MARY ELLEN O'HANLON

(Received for publication April 7, 1930)

In so far as the writer is aware, a description of the germination of the spores of *Reboulia hemisphaerica* has not been published. Spores for this study were collected before the middle of May at Sag Ravine, Cook County, Illinois, and in the latter part of the month at Sinsinawa Mound, Grant County, Wisconsin. The spore output in *Reboulia* is very small as compared with *Marchantia polymorpha* (3). The number of capsules to a head ranges from one to five (5) and the average number of spores to a capsule is approximated at 2500. The spores of *Reboulia* have already been described by Haupt (2) and by Miss Blair (1).

The results as here reported were obtained from two kinds of cultures, those in which the spores were sown on a mineral nutrient solution and those in which they were sown on porcelain plates kept moist with the same solution, a modification of Knop's mineral nutrient solution, in glass containers. All of the cultures were kept indoors in a room with north, south, and east exposures. Spores were thus germinated and young gametophytes developed in the spring, that is from a little after the middle of May until the middle of June, and from about the fifteenth of October until November fifteenth.

There is little or no change in the size of the spores (Pl. XLV, fig. 1), 70 to 80 microns in diameter, subsequent to their being sown until germination occurs. In this respect, as also in the paucity of spores, *Reboulia* resembles *Preissia* (3) and is in sharp contrast with *Marchantia polymorpha*, in which the spores, relatively very numerous and exceedingly small, about 18 microns in diameter, increase in size to probably eight times their original volume before germination occurs.

Spore germination is evident and, under favorable conditions of illumination and moisture, occurs in from two to five days. The spores seem to retain their viability for a considerable time as germination of spores about five and one half months old was probably one hundred percent. Spores a little older than these showed a low percentage of viability only, and germination tests made on spores seven to eight months old were totally negative.

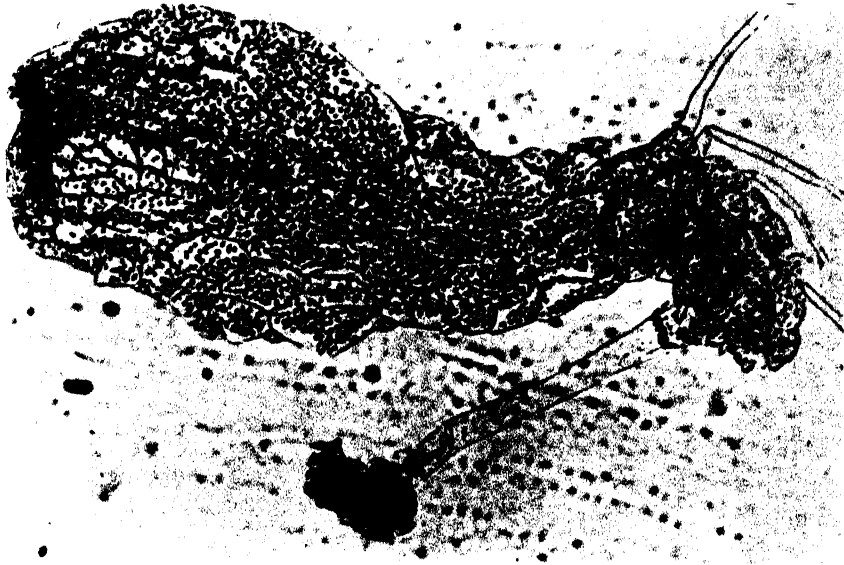
The first step in spore germination consists in the emergence of a germ tube with almost a simultaneous protrusion of a rhizoid more or less at right angles to the germ tube (figs. 2, 3). The nucleus, chloroplastids, and

oil droplets of the spore contents migrate to the tip of the germ tube, which is longer or shorter according to whether the light is weaker or stronger. No special difference seemed to result in the cultures of different daylight periods provided the intensity of illumination was adequate, except that it may be that those in the longer daylight periods reached a similar degree of development in a shorter time than was the case with those germinated in shorter daylight periods. When the spores sown upon the liquid medium remained upon the surface and were not too crowded, they developed in a manner not essentially different from those sown on a solid substrate, with the single exception that germination was probably a little more prompt on the liquid and, too, the liquid may have been a stimulus to greater germ tube length than was the normal case on the solid substrate.

The first cell is cut off at the end of the germ tube by a transverse wall (fig. 4). This division is followed by a second and sometimes by a third cross wall (figs. 5-7). The terminal cell divides by two divisions at right angles to each other, thus giving a primordial group of four cells at the apex of the germ tube (fig. 8). These four cells, constituting the growing point of the sporeling, divide by two cutting faces, thus forming a periphery of cells about the initial group (fig. 9). At this point secondary rhizoids are developed from cells in the primordial group and from cells in the periphery of this group (fig. 10). In lateral view the young gametophyte at this stage resembles an urn mounted on a columnar base, the column consisting of the germ tube and the cell or cells below the primordial group, and the urn is made up of the primordial group of cells and their peripheral outgrowth. In top surface view, this saucer-like group of cells shows a distinct apical region of meristematic cells (fig. 11). Subsequent to these preliminary steps, that is, from the point at which the apical region of meristematic tissue is developed, the general procedure in gametophyte development is quite similar to that in *Marchantia polymorpha* (3) and *Preissia quadrata* (4). The distinctive marks in gametophyte development in *Reboulia* seem to be the formation of the germ tube as an initial step and the development of the primordial group of cells at its tip.

Text figure 1 is a photomicrograph of a young gametophyte which was grown on a liquid medium. It was doubtless under a little more disadvantage as to illumination than other plants which were grown in the same culture, as is manifested by its greater relative length. This plant is, nevertheless, more or less typical in its general structure and more especially so in the sequence of events in its development. The germ tube is conspicuous and somewhat longer than it might have been under more favorable conditions of illumination; the primary and secondary rhizoids are typical, and the primordial group arose in the regular way. On the ventral side of the thallus in the midrib region, a rhizoid has budded off. It might not be expected that this organ of anchorage and absorption would appear on a plant grown upon a liquid surface. It may be that in the crowded culture

this plant was superimposed upon others and thus it received the stimulus for rhizoid development that it would have received on a solid substrate. The oil storage cells in the peripheral region of the thallus have no promi-



TEXT FIG. 1. Photomicrograph of a young gametophyte, $\times 250$.
(Photo by Robert G. Guthrie.)

nence in the accompanying figure since there was no osmic acid or other chemical used in this preparation which would bring them out.

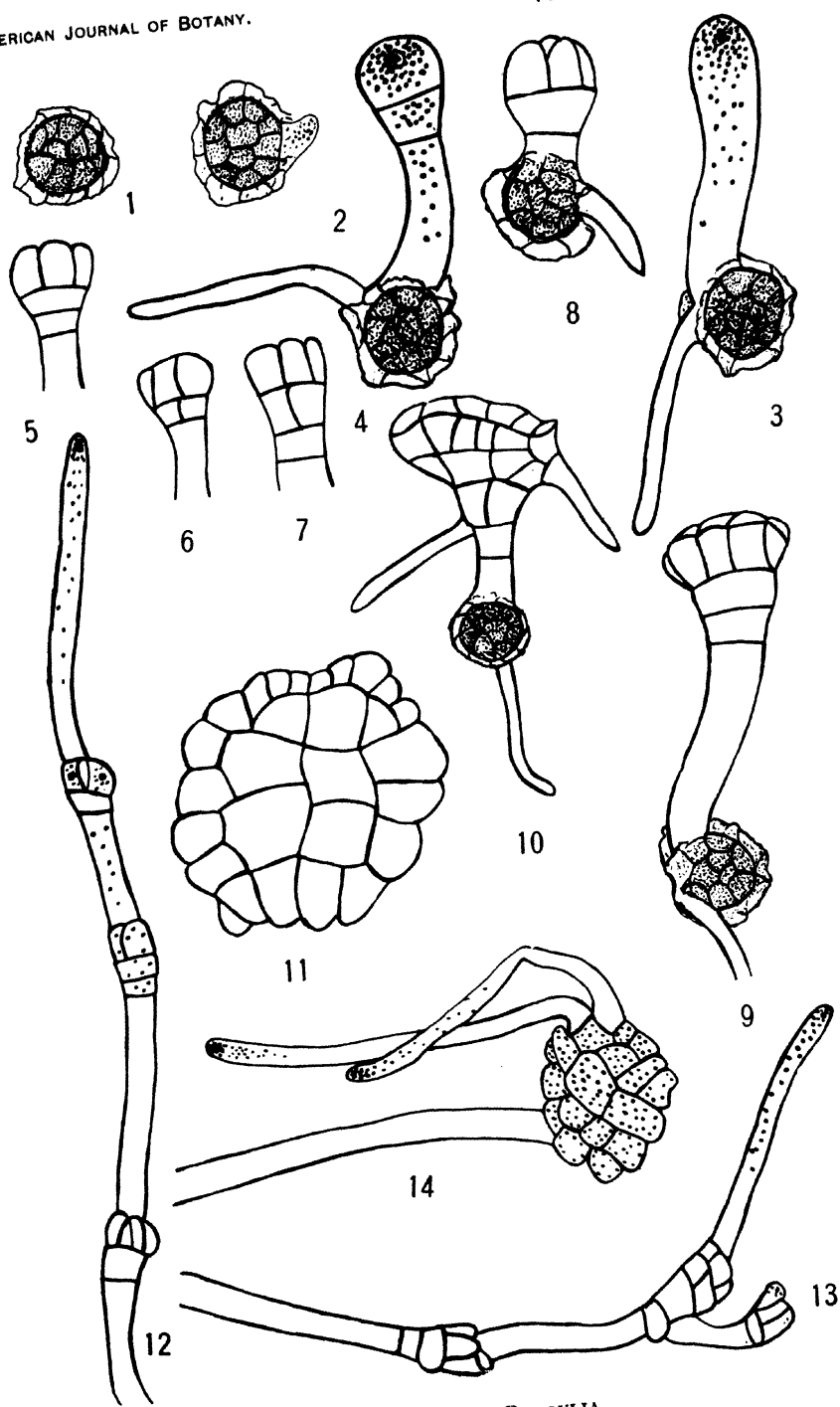
EFFECTS OF MOISTURE AND LIGHT

Reboulia as an adult plant is not only able to survive in a dry situation, but it fruits under these conditions as well (5). The thallus itself is much more conservative under these dry conditions, however, than it is in the more moist habitats. The germinating spores and the developing sporelings seem to adapt themselves to a hydrophytic habit. Their response to light conditions, however, is more definite. Figures 12 and 13 show the effect of weak illumination due primarily to over-crowding in the cultures; so much so, that these sporelings, if such they may be called, were much shaded if not completely covered by their more fortunate neighbors. Very long germ tubes obtained on both liquid and solid media when the light was inadequate. Secondary germ tubes, that is, tubes from the primordial group of cells developed at the tip of a primary germ tube, succeed each other by almost indefinite repetitions. Thus are produced, under weak light, alga-like structures. Figure 14 is a very bizarre form. This sporeling evidently made the usual start though with a handicap where light was concerned, as is manifest by the very long initial germ tube. It proceeded

according to the regular schedule to the initiation of the meristematic group of cells at the apex. Due to disturbance in the culture or to the more rapid growth of its fellows, this plant became under a disadvantage as to conditions of illumination, with the result that two of the cells in the marginal row were metamorphosed into functional germ tubes. Thus it seems that the germ tube may be of primary, secondary, or tertiary origin. That is, it may arise directly from the spore, it may develop from a cell in the primordial group at the tip of a primary germ tube, or, in the case of a tertiary germ tube, it may be the result of the metamorphosis of a cell in the meristematic tissue at the apex of a young gametophyte. This germ tube, of whatever origin, seems to be the *sine qua non* in the development of the young gametophyte in *Reboulia*.

SUMMARY

1. The number of capsules to a head in *Reboulia hemisphaerica* is from one to five. The number of spores in a capsule is estimated at approximately 2500.
2. The spores of *Reboulia* are 70 to 80 microns in diameter. They are one hundred percent viable for at least five months.
3. Spore germination occurs in about five days after the spores are sown. Fairly good light and plenty of moisture seem to be necessary for successful germination. Germination and the early stages in the development of the sporelings are about the same on either a liquid medium or on a solid substrate, provided the latter is sufficiently moist.
4. The first stage in spore germination is the emergence of a germ tube and the protrusion of a single rhizoid from the spore contents.
5. From the tip of the germ tube there are cut off by transverse divisions usually two, but sometimes three, cells.
6. The terminal cell of the germ tube then undergoes two divisions at right angles to each other, thus forming a primordium of four cells.
7. From this primordial group there is cut off a periphery of cells from the two outer faces of the primordial cells.
8. By differential growth in the saucer-like group of cells at the apex of the germ tube, a notch is formed in the marginal row of cells at the growing point. Subsequent development of the thallus is due to the activity of these meristematic cells in the marginal row.
9. Dorsiventrality of the young gametophyte is insured by the development of rhizoids on the ventral side of the thallus. These rhizoids mark the midrib of the older thallus.
10. Light of low intensity causes abnormal development, such as extremely long germ tubes or the rise of secondary and tertiary germ tubes.
11. No case was observed where a young thallus was initiated from any source other than from a germ tube.



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EXPLANATION OF PLATE XLV

The figures were drawn with the aid of a camera lucida. Most of the sketches were made from living material and for the rest, as well as for the photomicrograph, the sporelings were killed and fixed in chrom-acetic acid solution and stained in Delafield's haematoxylin.

FIG. 1. A mature spore. $\times 190$.

FIG. 2. A germinating spore. $\times 190$.

FIG. 3. Germinating spore showing germ tube and primary rhizoid. $\times 190$.

FIG. 4. Sporeling whose germ tube has undergone two transverse divisions. $\times 190$.

FIGS. 5-7. Germ tubes showing three or more division walls. $\times 190$.

FIG. 8. Young sporeling with primordium of cells at the apex of the germ tube. $\times 190$.

FIG. 9. Young gametophyte in which the primordial cells have divided. $\times 190$.

FIG. 10. Young gametophyte with lateral outgrowth from the primordial group. \times about 115.

FIG. 11. Surface view of young plant similar to that shown in figure 10. $\times 190$.

FIGS. 12-13. Sporelings grown in weak light and showing repetitions of secondary germ tubes. \times about 95.

FIG. 14. Surface view of a young gametophyte which when subjected to inadequate conditions of illumination developed germ tubes at its apex.

STUDIES IN THE CHYTRIDIALES IV. A FURTHER STUDY OF *DIPLOPHLYCTIS INTESTINA* (SCHENK) SCHROETER

J. S. KARLING

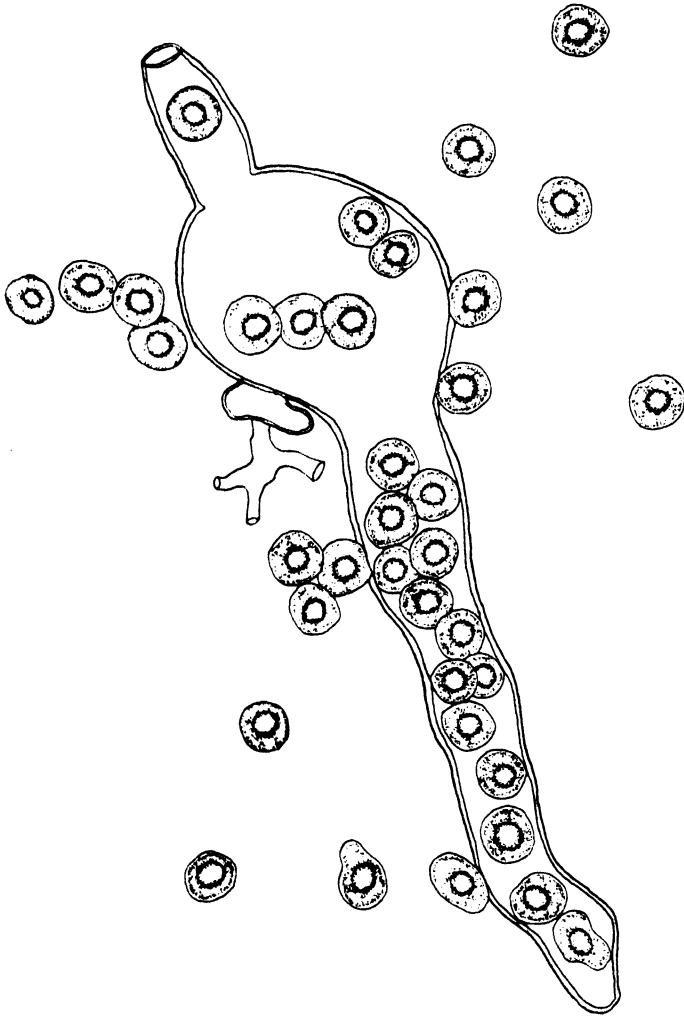
(Received for publication April 10, 1930)

In a previous study (1928) of *Diplophlyctis intestina* I have described in general the life history, pathogenicity, distribution, and occurrence of this organism in cells of American Characeae. More abundant and favorable material since that time has enabled me to follow more closely the consecutive stages in the penetration of the host cell and the development of the rhizoids, apophysis, and incipient sporangium. The additional data so far obtained are to a certain degree at variance with Zopf's (1884) and my own previous observations. The thickness and opacity of the Charophyta cell wall often renders observations of the early germination and penetration stages quite difficult and uncertain in living material. However, these stages have now been followed under more favorable circumstances in which the obscuring factors have been less prominent. The phases which I shall discuss here relate chiefly to the successive stages of zoöspore germination and the development of the apophysis and rhizoidal system.

GERMINATION OF THE ZOÖSPORES

As was previously described, the zoöspores germinate after they have lost their cilia and become quiescent. They may germinate either within the host or on its surface if they have previously escaped to the outside through the neck of the sporangium. In text figure 1 is shown a sporangium with two necks which had not penetrated the wall of the host cell. One of these necks is open while the other is closed. This sporangium was first discovered at 3:30 in the afternoon of February 24. Within the sporangium are 20 zoöspores which have lost their cilia, and close by on the outside are 17 others which have doubtless escaped from the same sporangium through the short open neck. Text figure 2 shows the same sporangium and zoöspores two hours later. All zoöspores with the exception of twelve have germinated. The condition in *Diplophlyctis intestina* is thus similar to that found in *Rhizophidium*, *Entophlyctis*, *Achlya*, and *Aplanes* in which the zoöspores may escape to the outside of the sporangium or germinate *in situ*. Nine of the twenty zoöspores within have germinated and the germ tubes have penetrated the wall of the sporangium. These germinating zoöspores continued to grow for nearly 13 hours, and I was fortunately able to follow their development to a very late stage. The successive stages of development of one of these zoöspores at different time

intervals is shown in Plates XLVI and XLVII. Figure 1 shows the beginning of germination at 3 : 40 P.M. The penetration tube is quite slender and pointed. It may often be as small as $1.5\ \mu$ in diameter in the beginning and

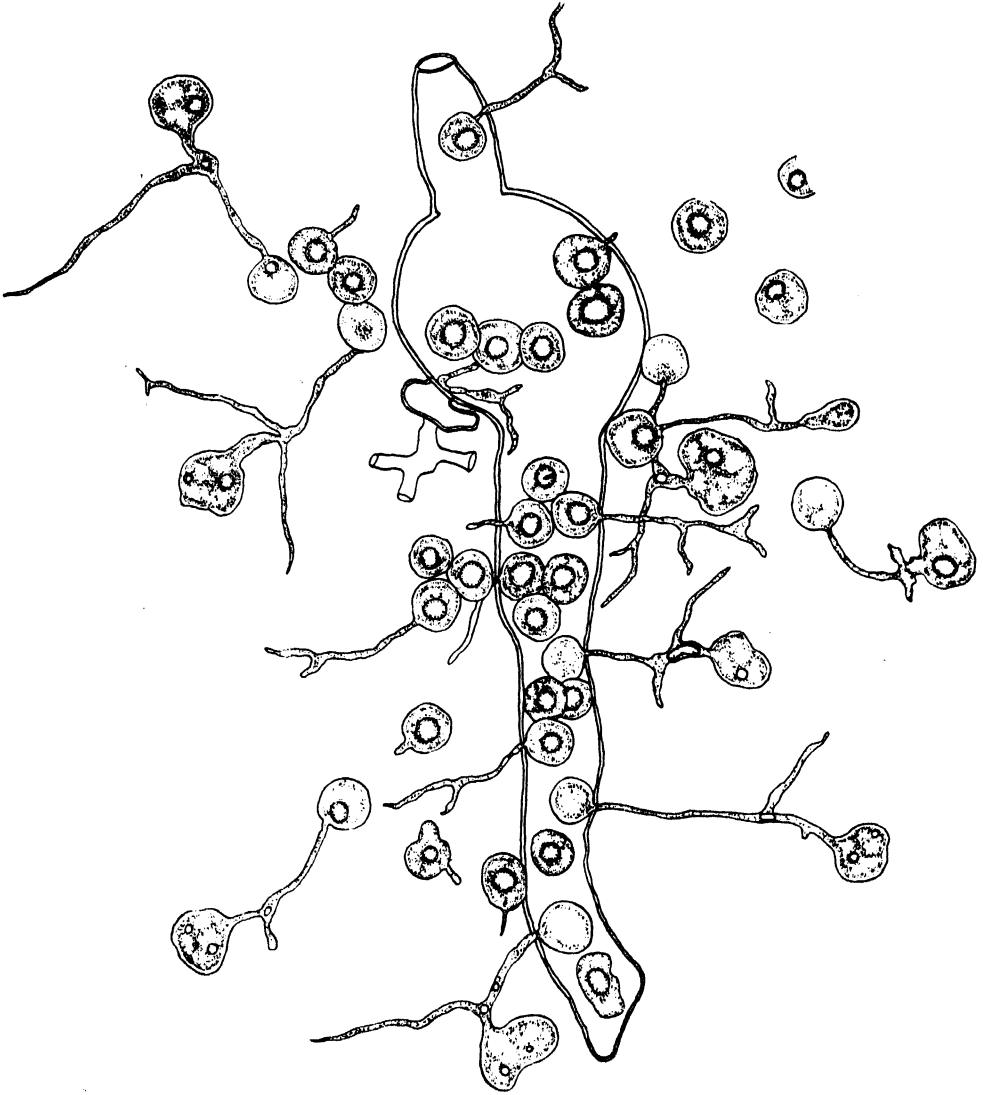


TEXT FIG. 1. A sporangium with two necks which did not penetrate the wall of the host cell. Within are 20 zoospores which have come to rest, and on the outside 17 additional zoospores which have apparently escaped through the short open neck.

then increase slightly as growth continues. Within 10 minutes (fig. 2), the penetration tube has almost doubled its length and formed three pseudopod-like outgrowths at its tip. As will become more apparent later, two of these structures are the rudiments of the rhizoidal system. The protoplasm in the zoospore itself has become somewhat vacuolated.

In figure 3 is shown the extent of development 30 minutes after germi-

nation began. The zoöspore has become more vacuolated and the refractive globule has moved near the region of the penetration tube. The two rhizoids at the end of the tube have elongated further, while the third pseudopod-like structure has become quite enlarged and globular.



TEXT FIG. 2. The same sporangium as in text figure 1 two hours later. All but 12 of the zoöspores have germinated, both outside and *in situ*. Differentiation into rhizoids and a globular part of the thallus is already visible.

Thus, at this early stage differentiation into rhizoids and a globular portion of the thallus is already visible. In text figure 2 are numerous other germinating zoöspores which show similar differentiation. Plate XLVI, figure 4, shows the condition at the end of an hour. There is little further

change with the exception of an increase in the length of the rhizoid rudiments and the diameter of the globular part of the thallus. In figure 5, made at 5 : 30 P.M., the changes are more marked. The spore is almost empty, the rhizoids are longer, and the refractive body has moved down the penetration tube and is about to enter the globular structure. This body may travel down *in toto* or break up into a number of smaller bodies. In this figure one small body remains in the spore, while another lies in the region of origin of the rhizoids. Its progress down the penetration tube is slow and may be observed with readiness. This slow movement suggests that there is an actual flow of the spore protoplasm through the penetration tube as growth continues. This is further suggested by the fact that the contents of the spore become more and more vacuolated, and finally nothing but the empty spore case remains.

The rate of growth of the globular portion of the thallus appears to be retarded to a certain degree at this stage. In figures 6, 7, and 8, drawn 1, $3\frac{1}{2}$, and $6\frac{1}{2}$ hours later, the diameter of this structure is approximately the same. The rhizoidal system, however, continues to develop extensively. As I have previously shown, a thallus whose diameter is no more than 12×10 microns may have a rhizoidal system whose radius is as much as 380 microns.

Figure 6 shows the beginning of a third rhizoid. The further growth of these rhizoids is shown in figures 7, 8, and 9. Plate XLVII, figure 9, shows the whole thallus 12 hours and 20 minutes after the beginning of germination. The spore is completely empty, the rhizoids have branched, and the apophysis has begun to develop.

As is shown in this figure, the apophysis begins as an enlargement between the point of origin of the rhizoids and the globular portion of the thallus. This place of origin is different from that which I previously observed and described. The material at hand at that time seemed to indicate that the apophysis arose as a swelling at the base of the incipient sporangium and that the rhizoids were subsequently developed from it.

The empty spore case is soon destroyed or collapses on the surface of the *Nitella* or *Chara* cell and the thickness and opacity of the host wall makes it very difficult to see the penetration tube and delicate rhizoids clearly. The general appearance of the young *Diplophlyctis* thallus minus the spore case and penetration tube suggests at once that the apophysis and rhizoidal system arise at the base of the incipient sporangium. Zopf figures (Pl. 8, figs. 2, 3, 4, and 5) and describes at length this manner of origin, and the early germination stages shown by Schenk (1858, figs. 12 and 13) suggest a similar origin. The development of the apophysis, as I have shown in figures 1 to 13, is thus similar to that described by Zopf for *Rhizidiomyces apophysatus*, *Rhizidium leptorhizium*, and *R. carpophilum*. In these forms the penetration tube first forms a branched rhizoidal system; then the external incipient sporangium begins to grow, and subsequently an apophysis

is developed as a globular enlargement inside of the host cell wall between the rhizoidal system and the sporangium.

The young thallus shown in figures 1 to 9 ceased development shortly after this figure was made, but a number of others continued to grow, enabling me to follow the sequence much further. Figure 10 shows a thallus with a well developed apophysis and rhizoidal system, which extended over a radius of $70\ \mu$. The spore case and penetration tube still remain attached. In figure 11 is shown a more mature stage in which the longest rhizoids extended for a distance of $90\ \mu$. Such stages as these show clearly the origin of the rhizoids and apophysis. The globular portion of the thallus which later becomes the sporangium is thus terminal instead of intercalary as is suggested in Zopf's and my own figures (1928, figs. 2 and 3).

The stages shown in figures 1 to 11 are of zoöspores which germinated and developed within the host cell, but I have also observed a similar development from spores which had germinated on the outer surface of the host cell. Figure 12 shows such a spore lying on the outside whose germination tube extends through the wall and which has developed a thallus on the inner surface of the *Nitella* cell. Sometimes the spores germinate and form long penetration tubes without any subsequent development of the thallus, as is shown in Plate XLVIII, figure 14. The penetration tube here shown has branched four times and extends to a distance of $120\ \mu$. It seems doubtful whether such a structure will ever give rise to a normal thallus.

I have also observed the growth of the penetration tubes from spores lying within the *Nitella* cell through the wall to the outside. Two such cases have been found in which the host cell was almost in the last stage of disintegration. Figure 13 shows a thallus in which the apophysis and rhizoids lie on the inner periphery, while the globular portion lies without. The appearance of this structure suggests that in germination the portion of the penetration tube beyond the point of origin of the rhizoids continued to grow within the layers of the wall until it reached the outside and there developed the incipient sporangium at its tip. I have never, however, seen such a sporangium come to maturity.

VARIATIONS IN SIZE AND SHAPE OF SPORANGIA

The type shape of the *Diplophlyctis* sporangium is oval with one neck for the escape of the zoöspores, but as I have already shown (1928, text fig. 2) the shape and size may vary to a notable degree. The variation in shape may often be so great that were it not for the presence of the apophysis and rhizoidal system and size of the zoöspores one might doubt whether the sporangia belong to *Diplophlyctis intestina*. Plate XLIX, figure 19, shows a sporangium which is very deeply and irregularly lobed, giving it a branched appearance. Its shape is so unusual that it is difficult to differentiate the sporangium from the necks. No less than eight elongated outlets are present, four of which have opened. This figure shows that the protoplasm in

the sporangial necks may undergo cleavage into zoöspores, and, furthermore, that there is little essential morphological difference between the necks and the sporangia.

In figure 17 is shown a small sporangium whose branched neck is more than seven times as long as its diameter. Such long necks have the appearance of very coarse irregular non-septate filaments as they are seen in the *Nitella* cells. In cases where the necks are so extensively developed the sporangium is relatively small in size. Figures 15 and 16 show two sporangia which are markedly lobed in shape.

The sporangial necks vary from the condition where they are indistinguishable from the sporangium to eight in number, as far as my observations go. They may either be simple or branched. Figure 20 shows a teapot-shaped sporangium with one short and two long necks. The sporangium shown in figure 21 has six necks. Figure 22 shows an unusual shape with a highly branched neck. The sporangium may also develop in such a manner as almost to envelop the apophysis, as is shown in figure 23. The apophysis itself may also become extremely irregular in shape and contour (figs. 15 and 16).

In 1890 Dangeard figured and described in addition to the ordinary resting spore a so-called oöspore which he believes is evidence of a primitive form of sexuality. In the formation of this oöspore the protoplasm of the sporangium condenses into a central mass and becomes surrounded by small oil globules. A membrane or wall is then formed cutting off a central spore from a periplasm. According to Dangeard, the periplasm disappears little by little as it is used up in the formation of the spines which cover the wall of the mature central spore. He did not observe antheridia, but described certain swellings on the oögonium which he believed might be vestigial antheridia.

I have observed three cases of internally formed resting cysts which may throw some light on Dangeard's observations. Figure 24 shows a fully formed resting spore with an apophysis and the remnants of the rhizoidal system within an empty sporangium. Its appearance is exactly similar to that of normally formed cysts and suggests that it has developed in the usual manner. This is further suggested by the occasional occurrence of young thalli within the sporangium (fig. 25) which appear to be developing normally in every respect. They have, doubtless, originated from zoöspores which germinated *in situ* without penetrating the sporangium wall, as is shown in text figure 2. It is quite probable that the resting spore or cyst shown in figure 24 has originated from such internally developed thalli. As the Charophyta cell decays and disintegrates the rhizoids are commonly broken off, and the cyst is left free with the apophysis attached. The hyaline apophysis is then very suggestive in appearance of a disintegrating antheridium.

MORPHOLOGY OF THE RHIZOIDS

Most systems of classification of the Chytridiales rest primarily on the presence or absence and the complexity of the rhizoidal system. The more extensive and complex the rhizoids the higher is the position of the organism in that system. Such a treatment, commonly found in textbooks on fungi, suggests and implies to beginning students a monophyletic origin, and that the mycelium of the higher fungi has gradually evolved from such rhizoids as are prevalent in the simple Rhizidiaceae through the more complex types of the Cladochytriaceae and Hypochytriaceae. On this view the fungous hypha is not the equivalent of an algal filament which has lost its chlorophyll. De Bary (1884), Brefeld (1889), Zopf (1890), Lagerheim (1893) and Petersen (1910) regard the Chytridiales as derivatives of the higher Phycomycetes and thus suggest that the rhizoids are degenerated mycelia. Zopf (1884), Schroeter (1897), Gwynne-Vaughan and Barnes (1927), and Gwynne-Vaughan (1929) regard them as mycelia; Fischer (1892), Atkinson (1909), Petersen (1910), and Gäumann (1925) call them rhizoids; while Nemec (1912) calls them haustoria.

Whether or not the rhizoids that are found in the Rhizidiaceae, Cladochytriaceae, and Hypochytriaceae are to be regarded as mycelia is obviously open to question. There are certain structural characteristics of rhizoids as they have been figured which in a general way, it seems to me, differentiate them from fungus mycelia. Almost at once rhizoids tend to decrease in diameter and run out to a fine point. The center of gravity of the growth process, so to speak, remains in the spore or in some immediate outgrowths of it like the sporangium or apophysis. The mature *Diplophlyctis* thallus as seen under low magnification bears a superficial resemblance to a germinating fungus spore, such as that of *Mucor mucedo*, for instance (Sachs 1882, fig. 174B), with its radiating mycelial filaments, but these mycelial hyphae soon carry the center of gravity to some new point where sporangia and zygospores are formed. That is, a true mycelium is a morphological unit constituting a vegetative stage as contrasted with the reproductive stage of the organism represented by sporangia, oogonia, etc. Growth of a fungus mycelium may thus be said to be apical to a certain extent. The cytoplasm at the apex is for quite a period more dense and less vacuolated in appearance, and the movement of accumulated foods is not backward to the spore.

In figure 18 is shown a portion of the rhizoidal system of a mature *Diplophlyctis intestina* thallus. The basal part near the apophysis is quite large in diameter with numerous fine vacuoles, but as the branches extend farther away from the sporangium they become less and less in diameter and finally run out to a fine point without showing any tendency to form new growth centers. Zopf's (1884) excellent figure 1, Plate 10, illustrates this further. This is also characteristic of the rhizoids of all of the Rhizidiaceae as they have been figured. Tapering of mycelial filaments, in *Sordaria* for

instance, also occurs at the edges of fungus colonies on culture media, but this is after the vegetative mycelium as a whole has been established. The rhizoidal system of *Diplophlyctis* is limited to the needs of the nutrition and development of the single sporangium. As I have pointed out before, the incipient sporangium may be rather small until the rhizoidal system has reached a very extended stage. Moreover, the rhizoids are nearly always pointed, even in the early stages of thallus development, so it is very doubtful whether this characteristic is an expression of thallus maturity in *Diplophlyctis*.

In the transition from the Rhizidiaceae to the Cladochytriaceae and Hypochytriaceae there is a very sharp difference in rhizoidal structure and development. In the first of these families each sporangium with its rhizoidal system is a separate and independent thallus, while in the latter two the thallus is duplicated to a certain extent as it spreads from one host cell to another. This is particularly true of *Cladochytrium* and *Urophlyctis* in which the portion of the thallus found in any one cell is almost an exact duplication of what is present in an adjacent cell. While it is difficult to draw sharp lines of distinction between rhizoids and mycelia and to say whether such differences are significant, it is well worth while to bear in mind such morphological differences in considering the phylogeny of the Chytridiales.

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DESCRIPTION OF PLATES

All figures were drawn from living material with the aid of a Spencer camera lucida and a Zeiss 2 mm. apochromatic objective N. A. 1.30, and compensating ocular No. 8.

PLATE XLVI

FIGS. 1-8. Successive stages in the germination and differentiation into rhizoids, incipient sporangium, and apophysis of a single zoöspore at different time intervals.

PLATE XLVII

FIGS. 9-11. Further stages in zoöspore germination and differentiation of rhizoids, apophysis and incipient sporangium.

FIG. 12. A zoöspore which has germinated on the outside of the host cell, penetrated the wall, and developed a thallus in a manner similar to that shown in figures 1 to 11.

FIG. 13. Portion of a young thallus which has apparently developed from a zoöspore which germinated within the host cell.

PLATE XLVIII

Fig. 14. A zoöspore which has formed an extensive and branched penetration tube without any subsequent thallus development.

FIGS. 15 and 16. Two unusually irregular and lobed sporangia.

FIG. 17. A globular sporangium with an extended and branched neck.

FIG. 18. A portion of the rhizoidal system of a mature thallus.

PLATE XLIX

FIGS. 19-23. Variations in the size and shape of sporangia and sporangial necks.

FIG. 24. A resting spore formed within the sporangium.

FIG. 25. Three young thalli within an empty sporangium.

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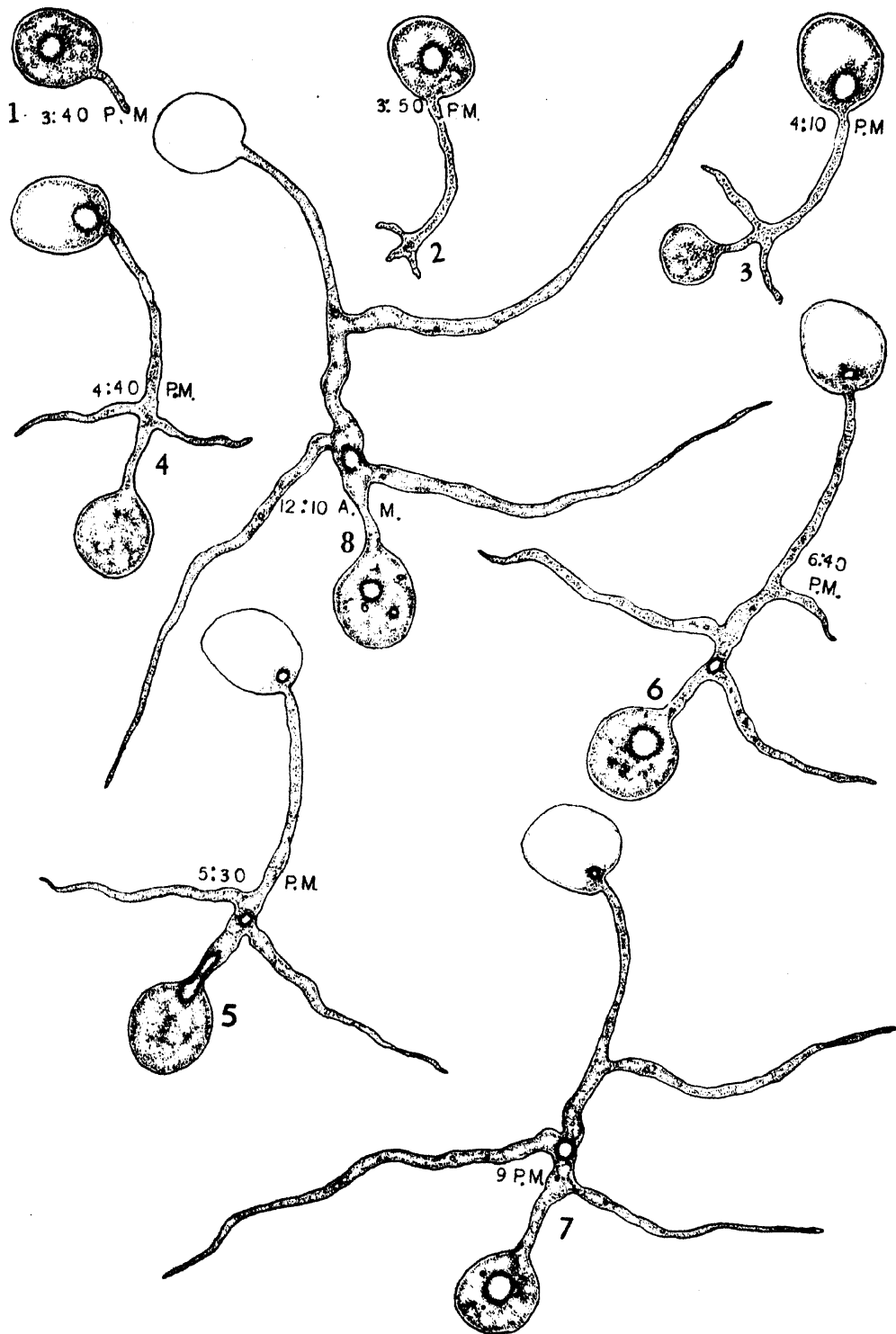
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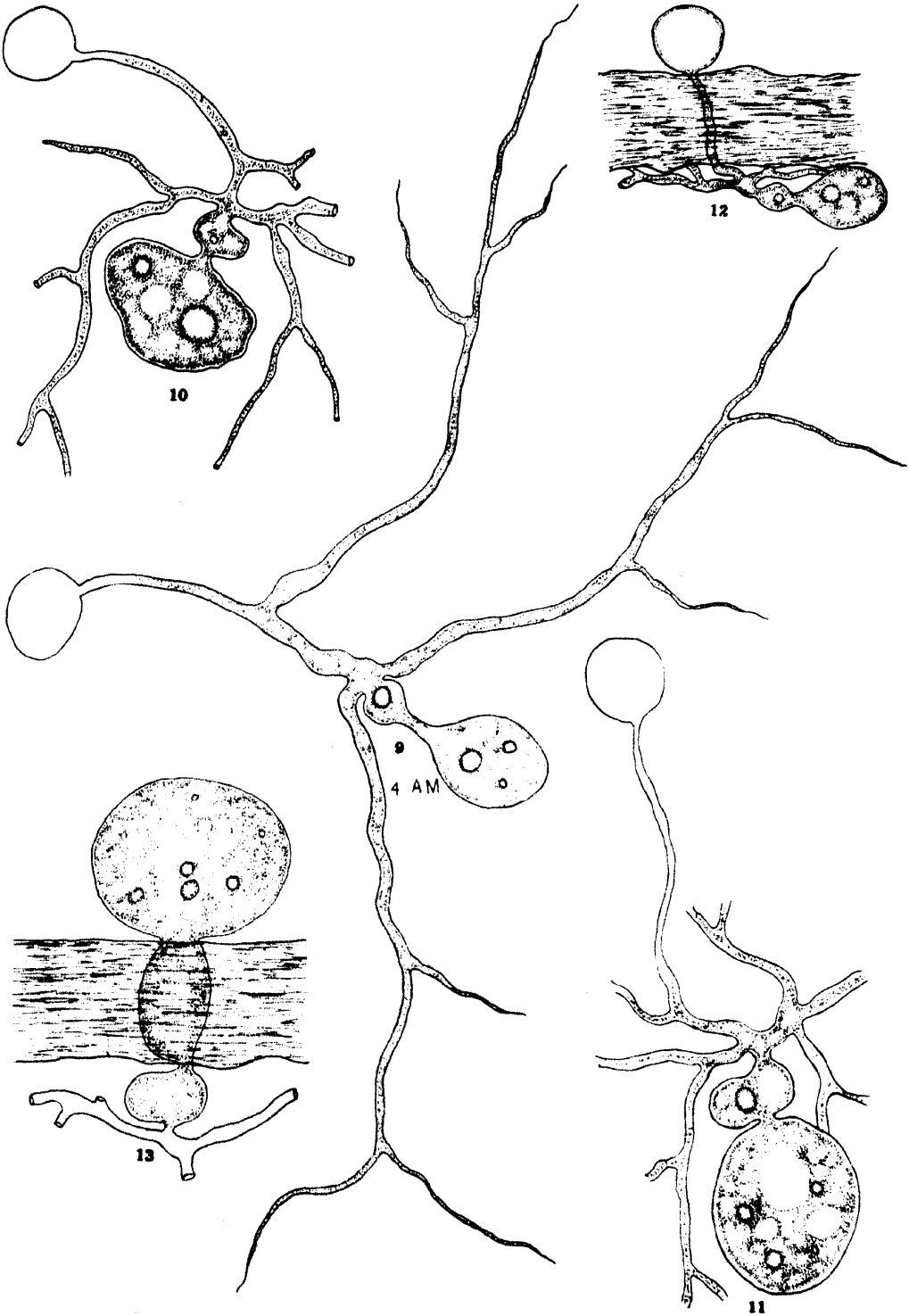
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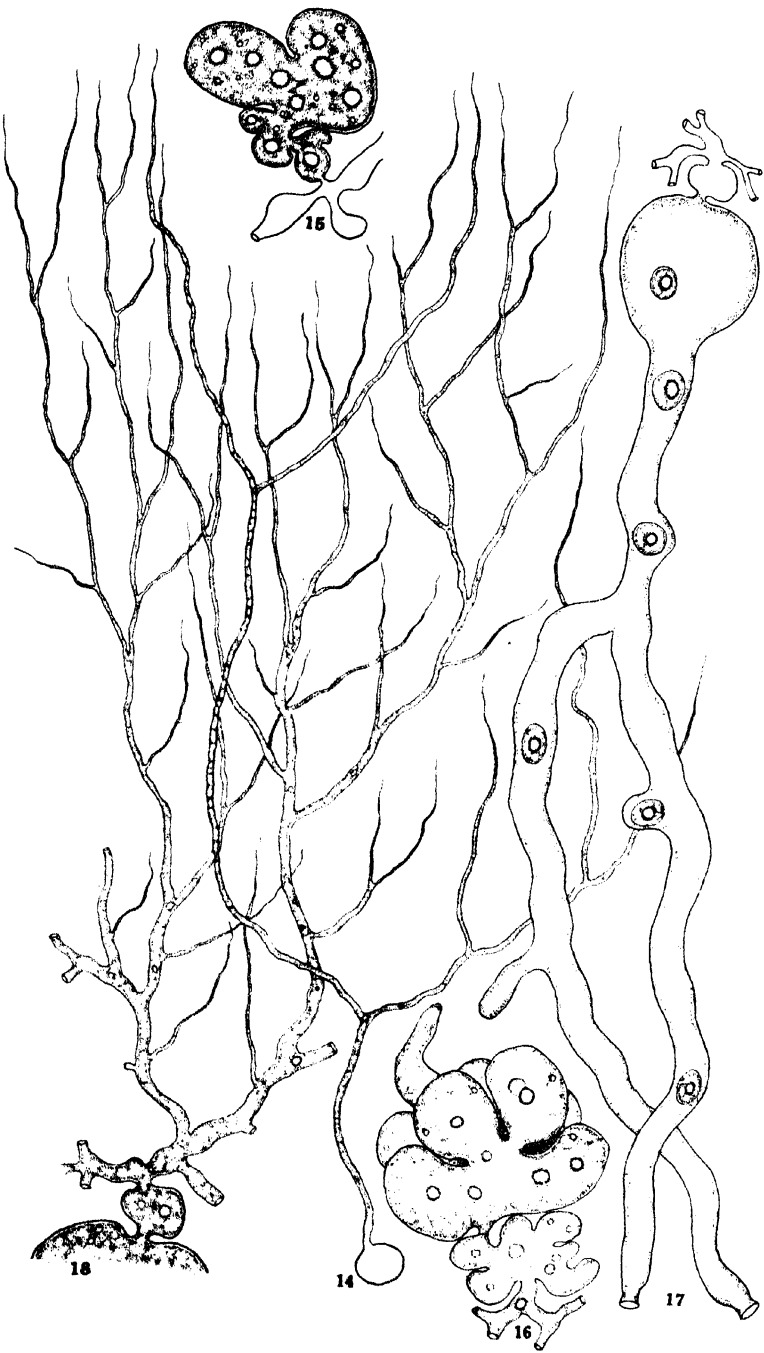
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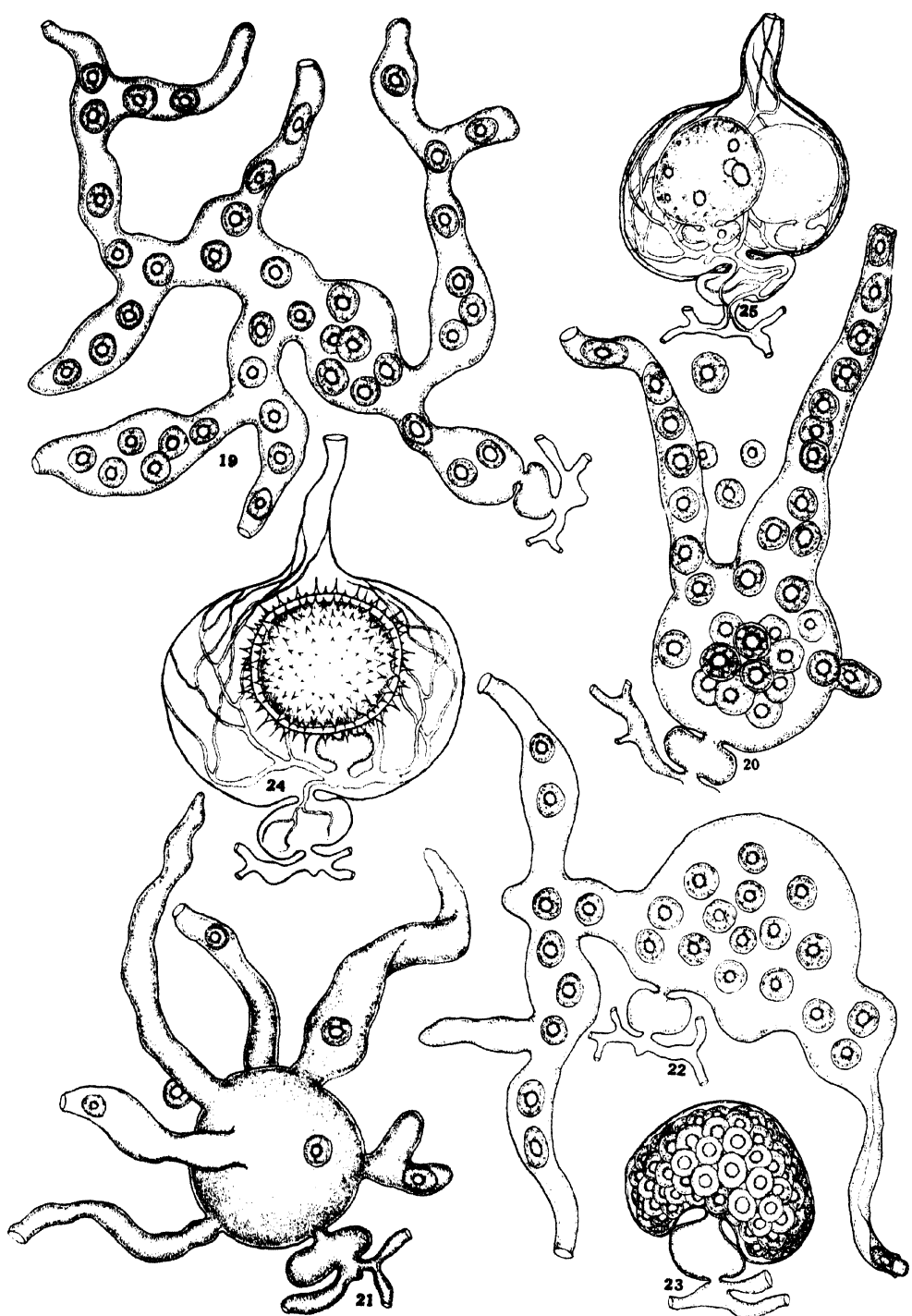
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COMPOSITION OF FUNGUS HYPHAE II. SCLEROTINIA¹

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INTRODUCTION

The genus *Sclerotinia* embraces a very important group of pathogenes. These have been recognized to cause serious losses to the fruit and vegetable industries, in the field, in greenhouses, and in storage, ever since it has been realized that fungi are capable of playing a parasitic rôle upon higher plants. Much attention has been given to studies of the morphology and physiology of various members of this group, but apparently very little interest, if any, has been shown regarding the structure and composition of the hyphae. It is the purpose of this investigation to endeavor to determine the nature of the cell-wall material, what it is and how it is arranged.

HISTORICAL

The absence of well defined layers of the material of which the cell walls of fungi are composed and the apparently inert condition of this material toward reagents such as dyes and even strong acids have presented difficulties which are very evident in the discussions of earlier investigators. It is also clear that a close analogy was anticipated between the structural substances of the higher plants and of fungi; yet when tests in current use to demonstrate the presence of cellulose, pectic compounds, cutin, etc. were applied to fungus hyphae, the results were negative or very indefinite.

The protecting influence of fatty substances such as fatty acids, with which the hyphae are impregnated, which was pointed out by the author (9) in a previous report and which is more strongly emphasized in this investigation, has not heretofore been fully realized. Such a condition may well be expected. Hansteen Cranner (2, 3) has shown that lipins and fatty acids are present in the plasma membrane, and fatty acids in the walls of higher plants. It has also been demonstrated by Lee and Priestley (5), in their studies of plant cuticle, that the movement of a fat compound in an aqueous medium depends upon its solubility. Accordingly, when fatty acids occur as sodium, potassium, or magnesium soaps they should move more freely than if they occur as calcium compounds.

Richter and Mangin both realized the complex character of the organisms which they studied. They suggested that the cellulose of fungi was infiltrated with albuminoid or nitrogenous substances which interfered with

¹ Published with the approval of the Director of the Ohio Agricultural Experiment Station.

the action of reagents and accounted for the failure to obtain definite results. Such a condition was found in the *Fusaria*. The outer covering of the hyphae appeared to be a protein-carbohydrate mixture, overlying a thin layer of cellulose which was well protected by a fatty acid. It was necessary to remove the fatty acid before the cellulose could be demonstrated.

In addition to these observations Mangin (6) was able to detect the presence of a new fundamental substance in the hyphae of many species of fungi which he studied. He called this substance callose. It was characterized as a colorless, amorphous substance insoluble in water, alcohol, and Schweitzer's reagent even after the action of acids; yet very soluble in weak, cold solutions of sodium and potassium hydrates, concentrated solutions of calcium chlorid, stannous chlorid, and sulfuric acid. In alkali carbonates callose is insoluble and in ammonia it swells to form a gelatinous substance. Callose is further characterized by its quality of fixing such stains as aniline blue and certain benzidine and toluidine dyes. Tswett (10) found that resorcin blue was very useful for detecting this substance.

Mangin reported callose to be generally distributed among different groups of fungi including the Peronosporaceae and Saprolegniaceae and various groups of Ascomycetes, and also in the Basidiomycetes. It seemed to be lacking in the mycelium of the Uredinales. He found this substance in the mycelial filaments of many groups of lichens as well as in certain algae. Even in the higher plants callose is a constituent of pollen grains, pollen tubes, and phloem cells. In the root hairs of many higher plants Howe (4) found callose instead of cellulose.

Mangin (6, 8) reported two different forms of callose. One could be demonstrated directly by definite staining and solubility reactions, whereas the other required treatment with caustic alkali (in alcoholic solution) or with oxidation reagents or both before it acquired the property of fixing dyes.

Although Mangin designates callose as one of the three fundamental substances found in the cell walls of plants, it must be admitted that we have as yet only a very limited number of tests at our disposal for the identification of this substance. It is very unfortunate that the last and best description of this carbohydrate was in connection with a very rare fungus, viz. *Bornetinia Corium*, described by Mangin and Viala (7), and apparently found only at one time in Palestine. Wisselingh (14), after referring to our limited knowledge of the actual chemical nature of callose, states that he prefers to consider this particular cell wall material as a hemicellulose. If we do not agree with Wisselingh we must refer to Mangin's report of 1890, or his later statement of 1910 based upon the research of Arnaud.

Other carbohydrates have been found in fungi. Winterstein (11), working with *Boletus edulis*, extracted a substance which he named para-dextran. He (12, 13) also isolated paraisodextran from *Polyporus betulinus*

and pachymose from *Pachyma cocos*. Upon hydrolysis all of these yielded glucose, but differed from cellulose by being insoluble in copper oxid ammonia, yet soluble in dilute alkalies. In the absence of uniform methods of extraction and purification one cannot form an opinion regarding apparent differences or similarities of the characteristics of these carbohydrates when compared with one another or with callose.

SPECIES INVESTIGATED

The following species of *Sclerotinia* were used in this investigation, pure cultures being provided by Professor H. H. Whetzel, of Cornell University.

1. *S. Duriaana* (Tul) Rehm, from *Carex*.
2. *S. Erythronii* Whet., from *Erythronium albidum*.
3. *S. intermedia* Ramsey, from salsify.
4. *S. minor* Jagers, from lettuce.
5. *S. Sclerotiorum* (Lib.), from tomato.
6. *S. Smilacina* Durand, from *Smilacina racemosa*.

Sclerotinia Sclerotiorum has been more generally known as *S. libertiana* Fcl. All of these organisms grew well upon liquid media, most of them at room temperature, and were found to be very satisfactory for this work. The species *S. Sclerotiorum* and *S. Erythronii* were used more than the others, because of their relatively large proportion of mycelial growth to sclerotia formation.

METHODS

The different representatives of *Sclerotinia* were grown upon Richard's solution in Erlenmeyer flasks of 250 cc. and 300 cc. capacity. Growth was allowed to develop from 4 to 6 weeks upon 100 to 125 cc. of liquid medium. At first some difficulty was experienced in obtaining prompt and rapid growth, due to the fact that the inoculum became immersed in the liquid, growing very slowly in freshly sterilized culture solution. This was thought to be due to a limited supply of oxygen. It was found that this difficulty could be overcome by placing the mycelial fragment on the side of the flask so that it merely touched the liquid.

Only fresh cultures were used in this work. The slimy, gelatinous nature of the outer covering of the hyphae rendered drying tedious, and there was no evidence that any advantage was gained by the use of dried material. Cultures which had reached the desired stage of growth were preserved in 70 percent alcohol until required for use.

Macrochemical procedure was supplemented by methods of chemical microscopy whenever there was an advantage in doing so. This frequently is the case in following the progress of solution and also for the detection of substances which are present in small quantities. The general plan consisted of resolving the hypha into the constituent parts of which it is composed by the use of appropriate solvents and of identifying or learning

the nature of these substances. *Sclerotinia Sclerotiorum* is taken as a representative for the group.

RESULTS

Fresh hyphae of *S. Sclerotiorum* manifest the typical inert condition regarding the fixation of dyes which one soon learns to anticipate when working with fungi. The outer covering is of a slimy, gelatinous nature. When treated with .5 percent ammonium oxalate or even hot water and filtered, and an equal volume of 95 percent alcohol added to the filtered extract, a small amount of gelatinous, stringy precipitate forms which collects at the top of the liquid. The alpha-naphthol test shows this precipitate to be a carbohydrate. Dilute ammonium hydroxid removes proteins, for the most part, together with a little of the carbohydrate. When an ammoniacal solution of cupric hydrate is used, only protein substance is detected in the extract. Weak solutions of sodium or potassium hydroxids (1-2 percent) appeared to remove larger amounts of carbohydrate than any of the other solvents employed, yet it was found to be a hopeless task to rely only upon these. Although each extraction yielded some precipitate, the amount was relatively small each time, varying in volume with the length of the period of digestion and the temperature.

When investigating the action of dyes upon the hyphae it was found that when fragments of mycelium were boiled for a time in 95 percent alcohol, then with ether, such stains as resorcin blue, thionin, analine blue, and congo red became fixed in the cell walls to some extent. The action of these dyes was further accentuated after treatment of the mycelium with boiling alcoholic potash (1 percent). These facts indicated that the presence of fatty substances, particularly fatty acids, intimately associated with the cell wall material, interfered with the action of dyes and of solvents. Acting upon this assumption the following procedure was adopted.

Fifteen mats of mycelium were washed with tap water to free them from the culture solution, followed by two changes of distilled water, and allowed to drain upon a wire screen to remove excess liquid. They were then ground in a food grinder. After grinding, the product was placed in a flask, covered with 1 percent ammonium hydrate, and allowed to stand over night. This liquid was filtered through muslin and replaced with fresh solution. After filtering, the residue was washed several times with hot distilled water to remove ammonia. The finely minced material was next refluxed with 95 percent alcohol for one hour, the hot alcohol filtered off, and the residue again covered with alcohol and refluxed. This was repeated until the alcoholic extract showed no trace of color. After the last extraction the residue was dried on a water bath until the alcohol was driven off, then placed in a flask and covered with ether. Usually it was necessary to digest three times with ether. The mycelium, free from ether, was next refluxed with 3 percent alcoholic potash until the liquid became dark brown in color. This was decanted and the extractions continued so long as the liquid

became colored. After the final extraction, the residue was pressed as dry as possible, placed in a flask, and covered with 400 cc. of 1 percent aqueous potash. After standing for 24 hours with occasional shaking, the liquid had become quite viscous, yet upon dilution, filtered readily. Upon acidifying the filtrate with hydrochloric acid a gelatinous precipitate formed, and when two volumes of alcohol were added, this collected as a voluminous, stringy mass at the upper part of the liquid and could readily be removed with forceps. Further extractions with aqueous potash yielded gradually decreasing amounts of this substance.

Although the fragments of hyphae appeared to be somewhat reduced in size when examined under a microscope, in general appearance and reaction toward dyes they were very similar to their original condition. It was thought probable that the alcoholic potash had penetrated only to a limited extent and that the portion of the hyphae remaining after the action of aqueous potash was still infiltrated and protected by fatty acid. Accordingly, the mycelium was again treated with alcoholic potash with the result that the liquid soon became brown, indicating that more fatty material was being removed. Examination of this extract showed that a fatty acid was present. It was thus found that all of the carbohydrate material could be removed by alternating extractions of the fungus with hot alcoholic potash followed by cold aqueous solution of the same alkali, usually one of the former to two of the latter.

After all of the carbohydrate had been extracted the fungus residue still retained its filamentous structure. It displayed no tendency to fix dyes, became yellow in color in the presence of iodine and sulfuric acid or chloro-zinc-iodid, and showed no reactivity when examined in polarized light. Since no evidence of the presence of cellulose was manifested, tests were made for chitin. A portion of the dried material was boiled in 40 percent potassium hydroxid for twenty minutes, then collected and hardened in 95 percent alcohol. This product gave the chitosan reaction and also the test for chitosan sphaerites.

The remainder of the residue was dissolved in concentrated hydrochloric acid with gentle warming. When completely dissolved the solution was diluted with distilled water, placed in a collodion sack, and dialyzed against distilled water. The water containing the diffusate was then concentrated to small volume and allowed to crystallize in a desiccator over sulfuric acid. When tasted the hard, glistening crystals which separated out were at first sweet, followed by a bitter saline after-taste. They were easily soluble in water, reduced Fehling's solution, and when warmed with sodium hydroxid, ammonia was formed. In appearance and reactions these crystals appeared to be identical with those obtained, when pure chitin was used, *viz.* glucosamine hydrochlorid. Accordingly the residual portion of the fungus was identified as chitin.

The Carbohydrate

The gelatinous, stringy precipitates obtained from the different extractions with potassium hydroxid had been collected and preserved under alcohol. Attempts were made to purify this substance by dissolving in 1 percent potassium hydroxid after the alcohol had been pressed out, acidifying with hydrochloric acid, and reprecipitating with alcohol. This process was repeated three times, after which the precipitate was thoroughly washed with alcohol to remove all trace of acid, finally with ether, and dried.

When prepared by this method the carbohydrate was almost insoluble in water, quite readily soluble in 1 percent sodium and potassium hydroxids after drying, yet before completely dry it dissolved much more quickly. It swelled in ammonium hydroxid and the alkali carbonates. The dry substance suspended in water was strongly doubly refractive in polarized light and readily fixed such basic dyes as resorcin blue, thionin and aniline blue, thus indicating that it was acidic in character. In glycerine heated to 280°, solution was prompt and complete. When fused with soda lime, ammonia was formed, and when ashed, a small residue remained. Examination of the ash revealed the presence of phosphorus. In order further to eliminate the possibility of the presence of inorganic phosphorus, 0.3 grams of the substance were dissolved in 100 cc. of a 0.5 percent solution of potassium hydroxid and dialysed against distilled water for 48 hours, the water being changed four times each day. Later it was electrodialysed for 12 hours, then evaporated to dryness upon a water bath at as low temperature as possible. This product contained only a trace of nitrogen yet apparently no decrease in the amount of phosphorus. The acidic nature of the carbohydrate as demonstrated by its capacity to fix basic dyes and the continued presence of phosphorus, even in thoroughly dialysed preparations, strongly indicated that an ethereal phosphate was combined with the carbohydrate molecule. In order to remove any possibility of the admixture of phosphatids, some of the pulverized substance was treated for a long time with boiling alcohol and hot ether and finally refluxed with 3 percent alcoholic potash, then taken up in distilled water and dialysed to remove alkali. After this treatment no change could be detected in carbohydrate. It was still doubly refractive, solubility and staining reactions were characteristic with phosphorus still present.

After the acidic properties of the carbohydrate had been established and accounted for, it was found that the substance could be precipitated out of potassium hydrate solution, as its copper salt, by adding copper sulfate. A 10 percent solution of the copper salt was used. This reagent was carefully added, avoiding excess, so as to precipitate all of the carbohydrate with the super-natant liquid still alkaline. This liquid was decanted or filtered, and the blue precipitate thoroughly washed with 0.5 percent potassium hydroxid, then suspended in 30 percent hydrochloric acid to remove the copper, one volume of alcohol added, and filtered. The precipitate was

washed several times with acid alcohol, then with alcohol until free of acid, and finally with ether. This product dried at 50° was snow white. The purified substance was very strongly doubly refractive, contained no nitrogen, gave positive tests for phosphorus, and otherwise showed reactions as previously noted.

Hydrolysis of the Carbohydrate

The product obtained by the copper-salt method of purification was used for this part of the work. A solution containing 1.6683 grams of this substance dissolved in 200 cc. of 0.5 percent sodium hydroxid gave a specific rotation of $[\alpha]_D = +65$. Two grams of the carbohydrate, finely pulverized, were triturated with 30 cc. of 75 percent sulfuric acid, then allowed to stand upon ice for twelve hours. Distilled water was added to make 500 cc. and the mixture refluxed for 4 hours. After neutralizing with CaCO_3 and evaporating to a syrup under reduced pressure it was found that all of the hydrolytic product was soluble in hot 95 percent alcohol. A solution of 1.15 grams of the syrup in 25 cc. of water showed a specific rotation of $[\alpha]_D = +52.57$. A phenylosazone melting at 206° C. was prepared. A portion of the syrup oxidised with HNO_3 , Sp. gr. 1.2, yielded saccharic acid, which was determined as its potassium salt. The sugar obtained by hydrolysis of the carbohydrate was thus identified as glucose.

Examination of the Alcohol and Ether Extracts

After the alcohol had been removed, this extract was treated with ether to dissolve fatty substances. The two ether extracts were then combined and the solvent distilled, leaving a dark brown oily mixture. When this was treated with acetone a part dissolved leaving the residue somewhat lighter in color. Some of the portion insoluble in acetone was fused with phosphorus-free sodium peroxid in a nickel crucible and tested for phosphorus by the ammonium molybdate method. A positive test was given. Another portion was placed in a small evaporating dish, 5 cc. of NaOH (36° Baume) and 20 cc. of water added. After boiling for a few minutes the mixture was acidified with 4 cc. of glacial acetic acid, boiled for about a minute longer, and filtered through a moistened filter. A drop of this filtrate was placed upon a glass slide with two drops of an iodine solution. When examined under the low power of the microscope, an ochre-colored precipitate was first noted. This soon changed to brown opaque crystals, known as "crystals of Florence" (1), which are the iodhydrate of choline iodid. The detection of phosphorus and of choline in the portion of the extract insoluble in acetone identified it as a lecithin.

The oily portion of the extract soluble in acetone was found to have an iodine value varying from 157.6 to 164.7. These values were obtained for fresh samples immediately after extraction. When kept in a desiccator over sulfuric acid for several months the iodine value became very much reduced, in one instance 63.1 being obtained.

DISCUSSION

The results of this investigation serve to emphasize the fact that the presence of fatty substances, particularly fatty acids, in the cell walls of fungi accounts for the apparent resistance or inactivity of the hyphae toward such reagents as alkalis, acids, and dyes. When these protective substances are removed the situation becomes changed. The reaction with dyes becomes possible, the use of solvents facilitated, and the hyphae can be resolved into component parts. It is well known that different groups of fungi show marked variation in composition, yet the protective rôle of fatty substances may well be anticipated in a very large majority of cases.

This fact accounts for the success which Mangin gained from the use of cold concentrated alcoholic potash. Treatment with this reagent mixture saponified the fatty acids to a limited extent, rendering them soluble in water, and thus permitting the action of dyes.

A reason can now be assigned for the use of bromine as outlined by Arnaud in Mangin's last report (8) upon callose. Mangin apparently was convinced that the substance which he found in *Bornetinia Corium* was callose, yet it seemed to be in a different form from that in which he had previously found it, being insoluble in dilute alkalis and incapable of fixing basic dyes. Upon treatment with bromine, Arnaud believed that the callose entered into combination with bromine, in which condition it became soluble in dilute alkalis. The results of this investigation point to a better explanation. The action of bromine upon the hyphae released the fatty acid combination, by brominating the fatty acids as well as other fatty substances, thus changing their solubilities. After this had taken place weak alkali readily dissolved the callose. This method was also tried upon cultures of *S. Sclerotiorum* and *S. Erythronii* with excellent results. The carbohydrate could be much more readily removed after the action of bromine than by the method outlined in this report. The bromine appeared to have very little if any effect upon the carbohydrate, all characteristics being retained. The use of bromine also aids in eliminating nitrogenous compounds.

The carbohydrate found in *Sclerotinia* agrees very closely with the substance which Mangin found in *Bornetinia Corium*, and designated as callose, a fundamental material which he maintained was present in a large number of species of fungi, frequently replacing cellulose.

Callose is described as being insoluble in water and dilute acids. This, however, is a relative designation. We do not know what criterion was used by previous investigators to determine this. The carbohydrate of *Sclerotinia* appears to be slightly soluble in dilute acids in the cold, and even water, at least it disperses sufficiently in the liquid to be filterable but not dialysable. The alpha-naphthol test indicates that the degree of solubility of 15 grams in 50 cc. of water is approximately of the same magnitude as 1 part of glucose in 10,000 parts of water. The presence of minute traces of sodium or potas-

sium hydrates changes the situation entirely. In alkaline solutions the carbohydrate is readily filterable but not dialysable.

SUMMARY

1. Analysis of the hyphae have been made of six species of *Sclerotinia*, and the same composition of cell structure has been found in all of the species investigated.

2. The outer covering of the hyphae was determined to be an acidic carbohydrate, which responded to such tests as are employed for the detection of callose. Underlying the callose was a basic skeleton of chitin.

3. The outer covering of callose was so thoroughly impregnated by fatty acid that it was inert toward the action of solvents and dyes until the fatty substance had been removed.

4. The hyphal walls could be resolved into their component parts by alternately extracting with hot alcoholic potash followed by cold aqueous solution of the same alkali.

5. The acidic character of the callose was determined to be due to an ethereal phosphate. Upon hydrolysis the carbohydrate portion yielded glucose, which was identified by its specific rotation $[\alpha]_D = +52.57$, oxidation product of saccharic acid, and the formation of a phenylosazone melting at 206°C .

6. The presence of the phosphoproteid, lecithin, in the alcoholic and ether extracts was demonstrated by positive tests for phosphorus and choline.

7. Chitin was identified by the chitosan reaction and also by the preparation of glucosamine hydrochlorid.

8. The action of bromine was found to be very effective in breaking up the callose-fatty acid combination.

I am greatly indebted to Professor H. H. Whetzel of Cornell University for furnishing me with the cultures of *Sclerotinia* used in this investigation.

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LOCAL AND SYSTEMIC INCREASE OF TOBACCO MOSAIC VIRUS¹

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INTRODUCTION

A number of investigators have studied the increase and spread of viruses in plants. Miss Purdy (7) described the production of mosaic virus in full-grown detached leaves of *Nicotiana tabacum*. Severin (8) determined the time required for a minimum amount of curly top virus to leave the inoculated area and pass a given distance in the sugar beet. McCubbin and Smith (5, 6) and Böning (1) made similar observations for mosaic virus in tomato and tobacco. Storey (9) made measurements of the same sort for streak virus in maize. Holmes (2) showed the time required for the production of mosaic virus in various concentrations in *N. tabacum*.

The development of a method of measuring virus concentration (3), by means of which large numbers of measurements may be made with comparative ease, has made it possible to secure further information in the case of tobacco mosaic disease. The purpose of this paper is to record the time required for the appearance of measurable amounts of tobacco mosaic virus at different distances from the site of inoculation in the inoculated leaf, in other leaves, in root and top, and in the stem of the plant; and to compare the subsequent increase of virus concentration in these parts with the increase at the site of inoculation.

METHOD

Plants of *Nicotiana tabacum* var. Turkish were inoculated with tobacco mosaic virus on restricted areas, as on single quarters of large leaves, to allow a study of the time elapsing before the appearance of measurable concentrations of virus at points distant from the site of inoculation, and of the rate of increase in concentration of virus at these points and at the site of inoculation. Samples of parts of these plants were collected at intervals, wrapped separately in small squares of cheesecloth, and pounded to express their juices. The juices were tested by a method described briefly in an earlier publication (3). This method takes advantage of the fact that *Nicotiana glutinosa* reacts to inoculation with tobacco mosaic virus by forming necrotic spots at the site of inoculation. Large numbers of these

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necrotic local lesions appear on the leaves of the *N. glutinosa* plants when they are inoculated with undiluted juice samples containing virus, and small numbers when the juice containing the virus has been diluted. To produce these lesions for use in estimating the relative concentrations of virus samples the following procedure has been uniformly used.

Plants of *N. glutinosa* were grown in four-inch pots until blossom buds began to form. The plants were trimmed by removing their growing tips and small upper leaves, as well as some old leaves near the base, leaving on each plant five large green leaves on a sturdy stem. Virus samples were prepared by pounding the pieces of tissue wrapped in cheesecloth. The moist cheesecloth was rubbed uniformly over the entire upper surfaces of the five leaves of the plant of *N. glutinosa*. The inoculated plant was then washed with water. It has been found that washing the plant with water after the inoculation never decreases the number of successful inoculations, and may increase the number, especially if the fluid sample containing the virus to be measured also contains some substances harmful to the tissues of the inoculated plant.

The lesions resulting from the inoculation appear after varying intervals of time. At 24° C. the inoculated leaves still appear green and normal after thirty hours; yet six hours later, only one and a half days after the inoculation, the first lesions are to be seen. At the end of forty-eight hours one half of the total number of lesions are visible, and after four days nearly all have appeared. Final results may be recorded on the fifth day at this temperature.

The purpose of this research was to determine the time of appearance of quantities of virus large enough to be easily measured at given points in inoculated tobacco and tomato plants. The technique employed was not suitable for showing whether or not very small traces of virus might have been present at these locations earlier, and hence did not result in a determination of the rate of spread in any location. The cutting experiments of McCubbin and Smith (5, 6) and of Böning (1) bear more directly on this phase of the question of the distribution of virus. These investigators cut away the inoculated portions and some inches of healthy stem at intervals to test whether or not virus had passed definite distances. The experiments reported in this paper show the presence of virus only after it has begun to increase in concentration in a given location. Successive measurements in each location tend to confirm each other and to indicate whether an increase in concentration occurs in each portion studied.

COMPARATIVE SUSCEPTIBILITIES OF *N. GLUTINOSA* AND *N. TABACUM*

In spite of the fact that only relatively large and increasing amounts of virus have been considered significant in the experiments reported in this paper, some criticism of the method of measurement might well be made if it were not first shown that the plant used for the measurements is reasonably

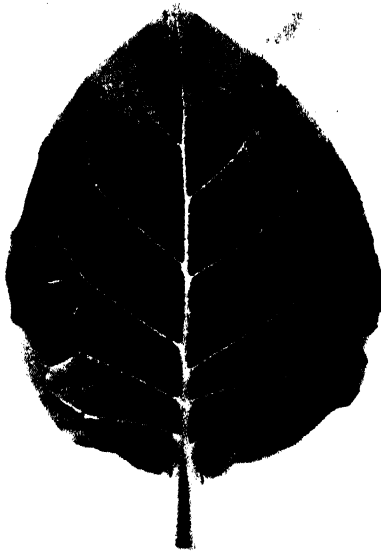
sensitive to inoculation. Since *N. tabacum* has been most commonly used for such measurements, the practical question is whether *N. glutinosa* is sufficiently susceptible to become infected by the rubbing method used in these experiments when the sample is so highly diluted that the presence of virus in it can be detected only with difficulty by means of *N. tabacum* with the same or other methods of inoculation.

An experiment was conducted to show the relations between the method used in these experiments and other methods. An estimate was made of the amount of water required to bring a frozen sample of virus to a strength sufficient to give an average of approximately ten lesions for each hundred leaves of *N. glutinosa* inoculated by rubbing. This dilution was one part of undiluted extract in one hundred and twenty-five thousand parts of water. Such a dilution was used to inoculate one set of *N. glutinosa* by rubbing, and four sets of *N. tabacum* var. Turkish by rubbing and by introducing fluid containing the virus into wounds made by scratching, crushing, and stabbing the leaves as described below. Thirty plants were used in each set. In the *N. glutinosa* set, which was inoculated by rubbing, twelve plants remained healthy, thirteen plants showed one lesion each, and five plants showed two lesions each. These two lesions were on different leaves of the plants in four of the five cases. Thus in this set eighteen plants in all produced one or more lesions. In the *N. tabacum* set, with plants of the same size rubbed with the same number of strokes with the same dilute virus sample, nineteen plants became diseased, and eleven plants remained healthy. Of the thirty *N. tabacum* plants scratched through the same sample of virus with twenty-five scratches each, only one plant became diseased. The virus used was so dilute (1 : 125,000) that with the small wounded area supplied by the scratches this result is not surprising. Similar results have been described previously (4) in comparing rubbing and scratching as methods of inoculation in *N. rustica*. Of the thirty *N. tabacum* plants inoculated by wetting the young leaves at the top of the plant and crushing these leaves with the fingers, seven became diseased and twenty-three remained healthy. Of the thirty *N. tabacum* plants punctured twenty-four times each in stem and petiole through a small wad of absorbent cotton wet with the same dilute virus, one plant became diseased and twenty-nine remained healthy. One hundred and fifty control plants, not inoculated but placed between the rows of the inoculated plants, all remained healthy during the course of the experiment. These tests indicate that rubbing is far more effective for inoculating *N. tabacum* than scratching, stabbing, or crushing, and that the difference of susceptibility of *N. tabacum* and *N. glutinosa* to rubbing inoculations with highly diluted virus samples is very slight. The *N. glutinosa* plants offer the advantages of distinguishing between single and multiple infections, of giving measurements over the wide range from undiluted extracts to dilutions of 1 : 1,000,000 without change of technique, and of reducing the probab-

ity of accidental transmission in the greenhouse, by reason of the very low virus content of *N. glutinosa* when infected.

SPREAD OF VIRUS WITHIN THE INOCULATED LEAF

The use of *N. glutinosa* for measuring the concentration of tobacco mosaic virus, with the method described above, made it possible to detect virus quantitatively even in small samples of tissue, and to repeat measurements frequently. It seemed possible, therefore, to attack the problem of the movement of virus from the inoculated portion of a leaf to other parts of the leaf, and to other parts of the plant. Quantitative measurements have not been reported previously to indicate whether or not virus spreads at once throughout the tissues of an inoculated leaf, nor has it been shown whether a large increase of virus occurs near the site of inoculation before it multiplies at the top of the plant where mottling appears.



TEXT FIG. 1. Leaf of *N. tabacum*, var. Turkish. Dotted lines show division into quarters, numbered as used in experiments on development of virus within the inoculated leaf, described in text and summarized in tables 1 and 2.

A number of plants of *N. tabacum* var. Turkish were studied in the following way to detect increase and spread of virus within the inoculated leaf if it should occur. Leaves not more than eight inches from the bases of stems of fifteen-inch plants were inoculated by rubbing extract of mosaic plants on one quarter of each leaf, the other quarters of the leaf being untouched. Two series of such leaves were studied, the inoculation being in a basal quarter in one series, and in an apical quarter in the other.

In the first series the leaves were inoculated in a basal quarter, indicated by number 3 in text figure 1. Care was taken not to inoculate the quarters represented by numbers 1, 2, and 4 in the diagram. Samples were examined at intervals to determine the concentration of virus in each quarter, similar quarters from three leaves being grouped to secure a large quantity for convenient testing. The midvein was discarded in all cases. The results of this experiment are shown in the first four columns of table 1. Virus

TABLE 1. *Measurements of Virus in Portions of Inoculated Leaf and in Distant Tissues of Tobacco Plants. Tests Made at Intervals After Inoculation of Leaf in Basal Quarter, Corresponding to Quarter Number 3 in Figure 1*

| Interval After Inoculation | Quarters of Inoculated Leaf | | | | Distant Parts of Plant | |
|----------------------------------|-----------------------------|-----------------------------|---------------------------------|----------------------------|------------------------|----------------------------|
| | Quarter No. 1, Apical | Quarter No. 2, Apical | Quarter No. 3, Inoculated | Quarter No. 4, Basal | Leaves of Top | Complete Root System |
| 1 day | 1 | 0 | 0 | 0 | 0 | 0 |
| 3 days | 0 | 0 | 142 | 0 | 1 | 0 |
| 5 days | 0 | 0 | 434 | 0 | 7 | 2 |
| 7 days | 0 | 0 | 1254 | 0 | 10 | 42 |
| 10 days | 0 | 0 | 1663 | 0 | 493 | 243 |
| 14 days | 0 | 0 | 621 | 44 | 219 | 110 |
| 21 days | 355 | 31 | 1741 | 433 | 527 | 317 |
| 28 days | 1138 | 750 | 746 | 412 | 769 | 1169 |

Measurements expressed in terms of number of lesions resulting from inoculation of *Nicotiana glutinosa* plants with extracts of tissues to be tested.

appeared in quantity in the inoculated quarter first. After two weeks measurable amounts of virus appeared in the opposite basal quarter, and subsequently in the two apical quarters of the leaf. The results of this experiment furnished evidence that virus may reach a high concentration near the site of inoculation before measurable quantities occur in distant portions of the leaf, but that distant parts of the leaf eventually contain virus.

In the second series the leaves were inoculated in one of the apical quarters of each leaf, indicated by number 1 in text figure 1. The quarters numbered 2, 3, and 4 in the diagram remained uninoculated in this case. The results are shown in the first four columns of table 2. In this case also the virus in the inoculated quarter soon reached a high concentration. After two weeks virus appeared in the opposite apical quarter of the leaf, which was the nearest of the three uninoculated quarters, and later in the two more remote basal quarters of the leaf. This confirmed the results of the first series, indicating a quick development of virus locally in the inoculated quarter of the leaf, and later slow passage to the uninoculated quarters.

TABLE 2.^a *Measurements of Virus in Portions of Inoculated Leaf and in Distant Tissues of Tobacco Plants. Tests Made at Intervals After Inoculation of Leaf in Apical Quarter, Corresponding to Quarter Number 1 in Figure 1*

| Interval After Inoculation | Quarters of Inoculated Leaf | | | | Distant Parts of Plant | |
|----------------------------|-----------------------------|-----------------------|----------------------|----------------------|------------------------|----------------------|
| | Quarter No. 1, Inoculated | Quarter No. 2, Apical | Quarter No. 3, Basal | Quarter No. 4, Basal | Leaves of Top | Complete Root System |
| 1 day..... | 0 | 0 | 0 | 0 | 0 | 1 |
| 3 days..... | 129 | 0 | 0 | 0 | 0 | 0 |
| 5 days..... | 304 | 0 | 0 | 0 | 0 | 0 |
| 7 days..... | 639 | 0 | 1 | 0 | 0 | 0 |
| 10 days..... | 677 | 1 | 0 | 0 | 349 | 179 |
| 14 days..... | 1168 | 25 | 3 | 0 | 805 | 414 |
| 21 days..... | 3848 | 207 | 188 | 375 | 1581 | 1337 |

APPEARANCE OF MEASURABLE CONCENTRATIONS OF VIRUS IN ADJACENT LEAVES

Quantitative measurements were made of the increase of virus in leaves adjacent to the inoculated leaf. Forty plants fifteen inches in height were inoculated with undiluted juice freshly extracted from mosaic plants. The inoculum was introduced into a single leaf about seven inches above the base of each plant by rubbing the leaf with cloth moistened with the extract. The potency of the inoculum was such that each leaf would receive much more virus than would be necessary to insure infection. At intervals a plant was examined by taking samples from the following parts: (1) its growing top, including all leaves less than an inch and a half in length, (2) the third leaf above the one inoculated, (3) the second leaf above the one inoculated, (4) the first leaf above the one inoculated, (5) the originally inoculated leaf, (6) the first leaf below the one inoculated, (7) the second leaf below the one inoculated, (8) the third leaf below the one inoculated, and (9) the lowest living leaf of the plant. At the beginning of the experiment these samples constituted practically the whole foliage of the plants. Later growth introduced numbers of leaves between the growing tip and the third leaf above the one originally inoculated.

The results of this experiment are summarized in table 3. A number of small observations, mainly to the number of ten or less, occurred in the early part of the experiment. These lesions may have been caused by virus from other sources necessarily handled during the experiment, or by virus left on the inoculated leaf after washing, and on other leaves of the plant from contact with water used to remove the excess of the inoculum. If the original mosaic extract in an experiment is dilute, such residues are very small and may not be detected. In the case under consideration the inoculum was concentrated, and large increases in the number of lesions in tests from a given location were necessary to ensure a significant determination of an increase of virus within the tissues of a sample.

TABLE 3. *Measurements of Virus in Inoculated Leaf, and in Leaves Above and Below Point of Infection in Tobacco Plants; Tests Made at Intervals After Inoculation*

| Time | Leaves at Top of Plant | Third Leaf Above Inocu- lation | Second Leaf Above Inocu- lation | First Leaf Above Inocu- lation | Inocu- lated Leaf | First Leaf Below Inocu- lation | Second Leaf Below Inocu- lation | Third Leaf Below Inocu- lation | Lowest Leaf of Plant |
|--------------|------------------------------|--|---|--|-------------------------|--|---|--|----------------------------|
| 0 days..... | 0 | 0 | 1 | 4 | 85 | 0 | 0 | 1 | 1 |
| 1 day..... | 0 | 0 | 0 | 0 | 217 | 0 | 0 | 2 | 1 |
| 2 days..... | 2 | 0 | 2 | 0 | 557 | 1 | 2 | 0 | 1 |
| 3 days..... | 19 | 0 | 0 | 0 | 802 | 0 | 0 | 0 | 0 |
| 4 days..... | 0 | 3 | 3 | 2 | 944 | 0 | 0 | 0 | 0 |
| 5 days..... | 0 | 2 | 0 | 1 | 936 | 9 | 0 | 2 | 1 |
| 7 days..... | 1 | 0 | 0 | 1 | 771 | 0 | 0 | 0 | 0 |
| 8 days..... | 306 | 0 | 7 | 5 | 1408 | 0 | 2 | 0 | 6 |
| 9 days..... | 2 | 6 | 0 | 1 | 1660 | 0 | 0 | 0 | 3 |
| 10 days..... | 1125 | 1 | 0 | 0 | 1069 | 0 | 0 | 0 | 85 |
| 12 days..... | 742 | 0 | 3 | 0 | 1530 | 1 | 1 | 3 | 162 |
| 14 days..... | 1920 | 156 | 0 | 1 | 2496 | 6 | 12 | 274 | 133 |
| 16 days..... | 1311 | 0 | 42 | 3 | 1205 | 5 | 45 | 175 | 164 |
| 18 days..... | 2086 | 0 | 0 | 4 | 2340 | 63 | 69 | 261 | 58 |
| 21 days..... | 763 | 184 | 73 | 20 | 1374 | 543 | 147 | 553 | 123 |
| 24 days..... | 943 | 264 | 426 | 262 | —* | — | — | — | — |
| 28 days..... | 1411 | 1563 | 667 | 653 | — | — | — | — | — |
| 35 days..... | 1100 | 278 | — | — | — | — | — | — | — |

* Dashes in table indicate that measurements were not made because of death of lower leaves.

As is shown by the table, significant increases of virus occurred in the inoculated leaf before virus appeared in measurable quantities in other portions of the plant. This furnished evidence, in addition to that given by the experiments on tissues within the inoculated leaf, that in the systemic mosaic disease of *N. tabacum* the first development of virus is at or near the site of inoculation, with no immediate detectable diffusion of virus to other parts of the plant. Later samples showed that virus appeared in the developing leaves at the tops of the plants about the eighth day, when symptoms were becoming visible there. Virus was still absent from the other leaves of the plants at this time, except in the case of the leaf originally inoculated. Subsequent samples showed an increase of virus in the lowest leaf on the tenth day, and eventually in all the leaves of the plants. The experiment was discontinued when the lower leaves had died off at the end of five weeks from the time of the original inoculation.

SPREAD OF VIRUS TO ROOT AND TOP OF PLANT

It was known from inoculation experiments that the roots of mosaic plants contained virus in quantities sufficient to allow transfer to healthy plants. It was not known whether this virus was concentrated or not. It was also of interest to know whether virus would appear in the tops of the plants before appearing in the roots, or whether the reverse would be true. Tests were therefore made of tops and roots of plants in which the movement of virus within the inoculated leaves was being studied.

The numbers of lesions resulting from the inoculation of test plants with juices from tops and roots are recorded in the fifth and sixth columns of tables 1 and 2, accompanying the results of inoculation of juices from portions of the inoculated leaves of the same plants. The samples from the tops contained the growing point and all upper leaves less than one and a half inches in length, together with the short portion of stem supporting these leaves. The root samples were cut free of stem material and washed thoroughly before being tested.

Both tables show that there was a large increase in virus concentration in the inoculated leaf before measurable quantities of virus were detected in tops or roots. Both tops and roots began to show virus at about the same time and the virus content increased at about the same rate in both locations. It has been believed generally that mosaic virus passes to the tops of plants before it goes to other parts. This belief was probably based on the early development of mottling in the top leaves. Although not accompanied by visible symptoms, movement of virus down the stem to the roots seemed to occur as early in these experiments as movement upwards. Further experiments on the spread of virus to roots and tops of inoculated plants are described in a later section.

CONCENTRATIONS OF VIRUS IN PETIOLE AND STEM

The distribution of virus in parts already mentioned was studied in relation to distribution in petioles and portions of stem. The results shown in table 4 confirmed earlier observations, and in addition showed that appearance of measurable quantities of virus in the petiole of the inoculated leaf and in the stem was much delayed in some cases.

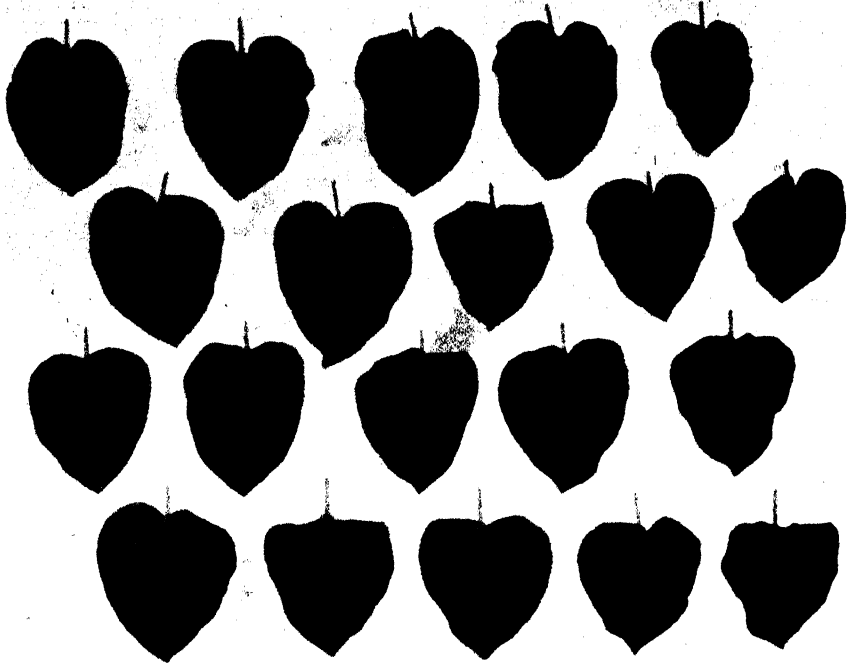
The experiment was carried out on plants which were about ten inches high when inoculated. Virus was introduced into one quarter of a leaf about half way up the plant. Each day a representative plant was divided into seventeen portions: (1) all top leaves one and a half inches in length or shorter, and the stem supporting them, (2) the second leaf above the one inoculated, (3) the first leaf above the one inoculated, (4) the first leaf below the one inoculated, (5) the second leaf below the one inoculated, (6) all leaves below these, (7) one basal quarter of the inoculated leaf, (8) the other basal quarter of the inoculated leaf, (9) one apical quarter of the inoculated leaf, (10) the other apical quarter of the inoculated leaf, including the whole inoculated area, (11) the petiole of this inoculated leaf, through which the virus must pass to reach the stem, (12) one inch of stem nearest to the petiole of the inoculated leaf, (13) two inches of stem just above this, (14) a second two inches of stem above, (15) two inches of stem just below the one-inch portion, (16) a second two inches of stem below, and (17) the roots washed free of dirt and carefully separated from the stem.

Virus appeared first at the site of inoculation, as it did in the experiments

TABLE 4. *Measurements of Virus in Leaves, Stem, and Root of Tobacco Plants at Various Intervals After First Introduction of Virus*

| Days after Inoculation | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 11 | 12 | 14 | 15 | 16 | 17 | 18 |
|--|----|----|-----|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|-----|
| All leaves at top of plant | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 672 | 2478 | 594 | 0 | 0 | 0 | 1007 | 352 | 715 | 724 |
| Second leaf above inoculation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 26 | 2 |
| First leaf above inoculation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| First leaf below inoculation | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 19 | 4 |
| Second leaf below inoculation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 18 | 77 |
| All leaves below this | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 21 | 8 | 32 | 58 |
| Left basal quarter of inoculated leaf | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 1 | 0 | 0 | 3 | 2 | 53 |
| Right basal quarter of inoculated leaf | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 42 | 1 | 4 | 55 |
| Left apical quarter of inoculated leaf | 2 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 5 | 450 | 425 | 9 | 45 | 39 |
| Inoculated quarter of inoculated leaf | 28 | 47 | 114 | 142 | 784 | 728 | 2398 | 2089 | 2840 | 2789 | 2500 | 1462 | 1836 | 1213 | 1318 | 1199 | 833 |
| Petiole of inoculated leaf | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 452 | 220 | 181 | 0 | 0 | 0 | 248 | 103 | 107 | 177 |
| One inch of stem nearest inoculation | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 220 | 363 | 481 | 0 | 0 | 0 | 337 | 260 | 336 | 287 |
| Two inches of stem above this | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 478 | 608 | 416 | 0 | 0 | 0 | 182 | 96 | 345 | 286 |
| Second two inches above | — | 0 | 0 | 0 | 0 | 0 | 0 | 546 | 772 | 323 | 0 | 2 | 0 | 310 | 299 | 369 | 562 |
| Two inches of stem below one-inch sample | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 456 | 640 | 401 | 1 | 0 | 0 | 241 | 289 | 251 | 274 |
| Second two inches of stem below | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 280 | 711 | 296 | 0 | 0 | 0 | 326 | 192 | 218 | 310 |
| Roots | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 219 | 471 | 647 | 0 | 0 | 1 | 381 | 481 | 499 | 324 |

on spread of virus within inoculated leaves. At this point, as may be seen in table 4, a significant increase in virus concentration was reached by the second day, the first day reading being too small to distinguish from a chance variation from the amount used locally to inoculate the leaf. A very high concentration was reached in the inoculated quarter before any virus appeared in the three uninoculated quarters. Text figure 2 shows the



TEXT FIG. 2. Twenty leaves of the test plant, *N. glutinosa*, used to measure the concentration of virus in a leaf of *N. tabacum* eight days after its inoculation in an apical quarter. Top row: five leaves of the test plant, showing more than two thousand lesions resulting from inoculation with juice expressed from the originally inoculated quarter of the leaf of *N. tabacum*. Other three rows: leaves of three similar plants of *N. glutinosa*, showing no lesions as a result of inoculation with juice from the three uninoculated quarters of the *N. tabacum* leaf. See table 4, sample for eighth day.

tests of virus concentration on the eighth day, the top row of five leaves representing the test of the inoculated apical quarter, the row next below representing the opposite and uninoculated quarter, the next row one of the basal quarters, and the lowest row the other basal quarter. As may be seen by reference to table 4, the inoculated quarter had produced virus, giving more than two thousand lesions on the five leaves of the test plant, whereas the other three quarters showed no evidence of containing virus on this day.

Subsequently virus was found in increasing concentrations in the locations in which it had been found in earlier experiments. The second

quarter of the inoculated leaf to show a significant increase was the opposite apical quarter about the fourteenth day. Increases of virus were found later in the two basal quarters of the leaf. Before virus had been found in other quarters of the inoculated leaf than the inoculated quarter, the tops of some plants showed symptoms, consisting of rolling and vein clearing of the developing leaves about the eighth day, and subsequent mottling. On the eighth day, when several plants had produced such symptoms, one of them was taken as a representative sample, although a number of plants had not yet shown any symptoms. The plant with symptoms in the top leaves showed the first observed virus in that location. It also showed virus in the petiole of the inoculated leaf, in the five stem portions, and in the roots. On the ninth and tenth days plants with similar symptoms were used as representative samples with the same type of results. At this time some of the plants still showed no symptoms. On the eleventh, twelfth, and fourteenth days these green plants were used as samples. It was found that virus was present in them in high concentration at the site of the inoculation, and in the case of the fourteen-day sample it was found that the virus had spread across the midvein of the leaf to the opposite apical quarter, but no virus appeared in the petiole of the leaf inoculated, in the five stem portions, in the root, or in the top of any one of these plants. Subsequent samples were taken from the mottled plants which soon predominated in the set. These finally showed virus in the old leaves of the plant, both above and below the inoculated leaf, as may be seen upon examination of the results recorded in table 4.

This experiment furnished information not given by former tests. Its measurements showed that virus was always present at the top of the plant when symptoms were in evidence there. When virus was present in the top leaves it was also present in quantity in the whole stem and in the petiole of the leaf inoculated. The appearance of measurable concentrations of virus in tissues of the inoculated leaf not more than an inch or an inch and a half from the site of inoculation required approximately as long a time as the appearance of virus at the extremities of the plant. This suggests that the movement of virus in midvein, petiole, and stem is far more rapid than movement of virus within the leaf blade. For example, on the eighth day after inoculation virus had passed into all portions of stem and into the roots, but was not found in any quarter of the inoculated leaf except the originally inoculated quarter.

The regularity of increase in the inoculated area shown in this experiment was in marked contrast to the irregularity in time of appearance in measurable concentrations in the stem of the plant. This regular increase of virus near the point of inoculation may be useful in the study of the influence of external factors on the virus in the plant.

It seemed possible that although no gradient of virus concentration was found in this case, one might be found if the length of stem were greater.

The rate of increase in concentration in any one portion of stem is rapid as soon as measurable concentrations of virus appear there. If the movement of virus along the stem were relatively slow, a gradient of virus concentrations would be expected. The following experiment was conducted to furnish a better test of whether such a gradient could be found.

Ten tall specimens of Bonny Best tomato, each fifty-nine inches high, were inoculated in leaves about twenty-seven inches from the base. A section of stem six inches long near this point was discarded from each specimen, and frequent tests were made of eight six-inch sections, four above and four below this. The results of the experiment are shown in table 5. It does not seem possible to detect a significant gradient of virus

TABLE 5. *Measurements of Virus in Stem of Tomato Plants at Various Distances From Point of Inoculation*

| Days After Inoculation | 0-6 Inches, Lowest | 6-12 Inches, Second | 12-18 Inches, Third | 18-24 Inches, Fourth | 24-30 Inches, Discarded | 30-36 Inches, Sixth | 36-42 Inches, Seventh | 42-48 Inches, Eighth | 48-56 Inches, Highest |
|-------------------------|--------------------|---------------------|---------------------|----------------------|-------------------------|---------------------|-----------------------|----------------------|-----------------------|
| 2 | 0 | 2 | 0 | 0 | — | 0 | 0 | 0 | 0 |
| 4 | 0 | 0 | 0 | 0 | — | 0 | 0 | 0 | 0 |
| 6 | 4 | 1 | 1 | 1 | — | 0 | 3 | 11 | 5 |
| 7 | 384 | 462 | 222 | 496 | — | 474 | 210 | 147 | 192 |
| 8 | 4 | 99 | 187 | 459 | — | 201 | 307 | 486 | 682 |
| 9 | 1388 | 1330 | 901 | 811 | — | 1093 | 627 | 654 | 724 |
| 11 | 417 | 592 | 489 | 528 | — | 513 | 519 | 543 | 711 |
| Total lesions | 2197 | 2486 | 1800 | 2295 | — | 2281 | 1666 | 1841 | 2314 |

concentration in the data. The initiation of the systemic infection in the individual plants was probably irregular, the eighth and eleventh day samples being less advanced than earlier ones, but the failure of any one portion of the stem to contain large amounts of virus earlier than others is the significant result of the experiment. This is well shown by the totals at the bottom of the table, since the presence of virus over a much longer period in one section of a stem than in another would have caused an increase in the total of the observations for that location. Apparently when virus reached the stem it was distributed so quickly that measurements made as in this experiment were not sufficient to show whether it became concentrated in one part before appearing in all others. The increase of the virus in all portions of stem seemed to be approximately simultaneous, so far as the data of this experiment show.

For comparison it was considered a matter of interest to know how rapidly virus would reach a measurable concentration in a stem not receiving virus from attached leaves. Internodal portions of tomato stem were removed from stems of Bonny Best tomato, inoculated with undiluted virus by means of thirty pin pricks made with No. 00 insect pins, and held each in a sterilized test tube at 25° C. Four pieces of stem were crushed each day and the juices tested on *N. glutinosa* plants. The increase of

virus in the stems is shown by the results recorded in table 6. It will be seen that the small amount of virus introduced by the five pin pricks multiplied rapidly enough to produce a significantly larger number of lesions by the third day. The stems then produced virus at a rate comparable to that previously shown in leaves. Since the virus increased in

TABLE 6. *Measurements of Virus in Detached Tomato Stems at Intervals After Inoculation. The Stems Were Inoculated After Removal From Plants*

| Time of Test | Four Comparable Series of Specimens | | | | Average of Four Measurements |
|-------------------|-------------------------------------|----------|----------|----------|------------------------------|
| | Series 1 | Series 2 | Series 3 | Series 4 | |
| 1 day | 1 | 7 | 1 | 0 | 2 |
| 2 days | 25 | 85 | 34 | 41 | 46 |
| 3 days | 465 | 280 | 115 | 87 | 237 |
| 4 days | 430 | 418 | 381 | 386 | 404 |
| 5 days | 490 | 433 | 565 | 342 | 458 |
| 7 days | 852 | 855 | 1183 | 590 | 870 |
| 10 days | 852 | 667 | 642 | 921 | 770 |
| 12 days | 1498 | 1762 | 2026 | 1037 | 1581 |
| 14 days | 1322 | 1038 | 775 | 1107 | 1060 |
| 16 days | 1597 | 1215 | 1036 | 1548 | 1349 |
| 20 days | 574 | 1037 | 1240 | 1070 | 980 |

concentration in these detached stems at about the same rate as shown in table 5 for the stems of plants with attached leaves containing virus, it seems probable that movement of virus in large quantities from these leaves to the stem need not be assumed in order to explain the increasing amounts of virus in the stem portions, although of course small quantities must escape to the stem to start the infection there.

FURTHER EXPERIMENTS WITH ROOTS AND TOPS OF PLANTS

In the experiment on the measurement of virus in tops and roots of plants inoculated in leaves about half way up the stems, and in the experiment on the measurement of virus in the stem after inoculation in leaves similarly situated, the evidence favored the view that the movement of virus was so rapid up and down the stem, that virus did not become noticeably concentrated in the portions of the stem near the point of inoculation before the time when the extremities of the plant also contained virus.

Another experiment with *Nicotiana tabacum* was made to determine whether the introduction of virus in an upper leaf would result in the appearance of virus in tops before roots; and to determine whether the introduction of virus in a lower leaf would result in the appearance of virus in roots before tops.

Plants were grown to a height of fifteen inches. In one series virus was introduced into a leaf not yet fully developed, about two inches below the top of each plant. The inoculated leaf, its petiole, the top one inch of the stem with its attached leaves, and the washed roots of plants were tested

at intervals, as indicated in table 7. Significantly increased amounts of virus appeared in the inoculated leaf on the second or third day. In this set of plants inoculated in a young leaf, virus appeared in the petiole of the inoculated leaf, in the top, and in the root on the fourth day. Symptoms of the systemic disease appeared on these plants on the sixth day.

TABLE 7. *Measurements of Virus in Different Parts of Tobacco Plants at Intervals After Inoculation in Young Leaves*

| Time | Leaf Inoculated | Petiole of Leaf | Top of Plant | Roots of Plant |
|------------------|--------------------|--------------------|-----------------|-------------------|
| 1 day | 0 | 0 | 0 | 0 |
| 2 days | 23 | 0 | 0 | 0 |
| 3 days | 89 | 0 | 1 | 0 |
| 4 days | 770 | 42 | 56 | 23 |
| 5 days | 1270 | 355 | 1232 | 150 |
| 6 days | 1414 | 108 | 575 | 227 |
| 7 days | 1162 | 812 | 625 | 159 |

In the second series virus was introduced into an old leaf about two inches above the base of each plant. The two series were carried out simultaneously and on comparable plants. Samples were taken of the inoculated leaf, its petiole, the top one inch of the stem with its attached leaves, and the washed roots of plants. Results are recorded in table 8. Significantly increased concentrations of virus appeared in the inoculated leaf on the third day, but not in the petiole of the inoculated leaf, the tops, or the roots during the first twelve days after inoculation. The rate of increase of virus in these old leaves seemed to be approximately as great as that in the young leaves of the other series, but movement from the old leaf to other portions of the plant was delayed. On the sixteenth day two cases of the systemic disease appeared among the five remaining plants of this series. On the next day one more case appeared clearly, and of the remaining two plants one showed the very faintest detectable flecks of yellow in its new leaves. This doubtful case was examined by inoculating juices from its tissues as before. It showed virus in all the parts tested, thus giving evidence that even in this early stage of spread of virus from an old leaf located near the bottom of the plant, virus was in evidence in the distant top as well as in the nearby roots.

The roots showed an unusually high result in this case, which might have led to the conclusion that the inoculation of a leaf near the base of the plant allowed virus to reach the roots before reaching the top, but a more critical experiment showed that this was not true in general. The experiment was arranged to obtain adequate numbers of records at the critical moment, when for the first time virus was present in parts at a distance from the inoculated leaf. A set of plants like those used previously was inoculated as before in an old leaf about one inch from the base of each plant. Instead of testing the plants daily as in the previous experiment,

the plants were left in a greenhouse for ten days, when symptoms were visible in the tops of one-fourth of the plants. The plants with symptoms were discarded. Former experience indicated that most of the remainder of the set would have shown symptoms within a day or two if allowed to remain. Ten representative plants were tested at once in the hope of finding all of the early stages of spread of virus in them. The results are shown in table 9. Two plants gave no evidence of the presence of virus

TABLE 8. *Measurements of Virus in Different Parts of Tobacco Plants at Intervals After Inoculation in Old Leaves. Compare With Table 7*

| Time | Leaf Inoculated | Petiole of Leaf | Top of Plant | Roots of Plant |
|-------------------|--------------------|--------------------|-----------------|-------------------|
| 1 day | 1 | 0 | 0 | 0 |
| 2 days | 6 | 0 | 0 | 0 |
| 3 days | 344 | 0 | 0 | 0 |
| 4 days | 431 | 0 | 0 | 1 |
| 5 days | 482 | 0 | 1 | 0 |
| 6 days | 1439 | 0 | 6 | 0 |
| 7 days | 904 | 0 | 5 | 1 |
| 8 days | 1050 | 0 | 0 | 0 |
| 9 days | 1310 | 0 | 0 | 0 |
| 10 days | 1067 | 0 | 0 | 0 |
| 12 days | (Leaf dead, 474) | 0 | 0 | 0 |
| 17 days | (Leaf dead, 578) | 359 | 517 | 806 |

TABLE 9. *Measurements of Virus in Different Parts of Tobacco Plants Ten Days After Inoculation in Old Leaves. None of the Plants Showed Symptoms When Tested*

| Plant | Leaf Inoculated | Petiole of Leaf | Top of Plant | Roots of Plant |
|-------|--------------------|--------------------|-----------------|-------------------|
| No. 1 | 1407 | 0 | 0 | 0 |
| 2 | 1035 | 0 | 0 | 0 |
| 3 | 887 | 1 | 0 | 7 |
| 4 | 1296 | 7 | 4 | 3 |
| 5 | 1305 | 46 | 92 | 4 |
| 6 | 648 | 42 | 81 | 70 |
| 7 | 583 | 155 | 50 | 8 |
| 8 | 1123 | 81 | 307 | 107 |
| 9 | 1144 | 177 | 976 | 26 |
| 10 | 794 | 522 | 697 | 700 |

except in their inoculated leaves, a third showed none in the top but such small concentrations in roots and petiole that the single negative result in the top did not constitute significant evidence against the presence of an equal amount there, and seven showed virus in all parts tested. This series of measurements gives more data than are given in table 8 on plants studied at the time of the first appearance of measurable amounts of virus at the extremities of the plant. When the results are considered as a whole they seem to furnish no evidence of appreciably earlier or later arrival of virus in roots than in tops.

A striking result of this set of experiments was the evidence that virus developing at approximately equal rates in young and old leaves, and coming to equally high concentrations in both, might spread from the young leaves to distant parts of the plants many days earlier than from the old leaves.

DISCUSSION

Lesions resulting from the inoculation of plants of *N. glutinosa* increase in number as the concentrations of virus in the inoculated extracts increase, but the ratio between the number of lesions and the concentration of virus is a variable which decreases as both of these increase. That is, the number of lesions does not bear a constant relation to the concentration of the virus; just as the number of colonies produced upon pouring plates from a series of bacterial suspensions does not bear a constant relation to the number of bacteria in the original series of suspensions. Since it is customary to think of the number of bacteria in terms of the number of colonies produced on pouring plates, it may be permissible to record increases in mosaic virus concentration in various parts of the host plant in terms of the increase in the number of lesions produced upon the inoculation of extracts of samples of tissues.

It is possible to interpret the results of measurements of virus concentration expressed in terms of local lesions by means of previously established dilution tables, which show the relation between known water dilutions of virus and the number of lesions produced in *N. glutinosa*. A graph giving this information has been presented in an earlier paper (3). The samples measured in the work described in this paper were whole juice samples, and their interpretation by means of the results of inoculation with water dilutions may be open to criticism. Therefore, the number of lesions is presented in the tables of this paper without conversion into terms of relative virus concentration; but in such cases as this, in which the inoculated juices are not known to be seriously harmful to the cells of the test plant, such a conversion of the data might perhaps be made with propriety if it were desired to represent the relative concentrations of virus in different parts of host plants.

SUMMARY

By means of measurements made with *Nicotiana glutinosa*, the concentrations of virus in portions of inoculated plants of *N. tabacum* var. Turkish were studied, and it was found that:

1. Mosaic virus developed to a high concentration near the site of inoculation in a leaf of *N. tabacum* before reaching measurable concentrations in other portions of the inoculated leaf or in other parts of the plant.
2. A slow spread of virus through the tissues of the inoculated leaf accompanied the increase in concentration near the site of inoculation, and appeared to be independent of the rapid spread which carried virus to distant parts of the plant. This local increase and slow spread of the virus constitute a local primary phase of the disease.

3. The systemic or secondary phase of the disease was marked by the nearly simultaneous appearance of increasing quantities of virus in the petiole of the inoculated leaf, in all portions of the stem, in the developing top leaves of the plant, and in the root, with later invasion of old leaves.

4. In a series of plants all successfully inoculated in similar leaves at the same time, the local increase of virus within the tissues of the inoculated leaf blade occurred simultaneously in all plants; but systemic spread of virus, with its attendant mottling of developing leaves, occurred early in some individual plants and late in others.

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SUCROSE AND STARCH CHANGES IN POTATOES TREATED WITH CHEMICALS THAT BREAK THE REST PERIOD^{1, 2}

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According to previous experiments (2) the treatment of dormant potato tubers (*Solanum tuberosum* L.) with chemicals such as ethylene chlorhydrin and sodium thiocyanate greatly increased the sucrose content of the tissue. However, the comparisons were made merely between untreated potatoes and those treated with concentrations of chemical which were approximately optimum for inducing sprouting. No intermediate concentrations were tested. Consequently, it seemed that a study of the effect of suboptimal amounts of chemical would give more conclusive evidence regarding the reality of the sucrose increase, and, furthermore, would be of interest in showing the general relation between the concentration of chemical and the amount of the gain in sucrose.

In 1929 press-juices were obtained from potatoes that had been treated with amounts of chemicals increasing by steps from zero up to approximately the optimum. Ethylene chlorhydrin, sodium thiocyanate, and thiourea were included in the tests and the enzym activities of the juices were measured and reported upon in a previous paper (3). Samples for sugar analysis were also taken and the results here reported show that the relation between the concentration of chemical and the amount of sucrose found in the juice was very close, the sucrose increasing with the increase in chemical and giving in nearly all cases a series of sucrose readings corresponding to the concentrations of chemical used in treating the potatoes.

In the previous report (2) it was shown that sucrose increases were observed in lots that had been sampled only 48 hours after treatment, and it was stated that sampling would need to be started at an earlier period in order to learn the time of the initiation of the sucrose change. The experiments here reported upon show that the time at which increases in sucrose were observed varied with different experiments, and was as early as 24 hours and as late as 72 hours.

Finally, in the previous report it was shown (2, p. 332) that the starch content was found to have been decreased by the treatments, provided the tissue for the analysis was taken at the eyes of the tuber, but that if the balance of the tissue (seed-piece minus eye-tissue) was sampled the question

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of a difference between treated and check was somewhat in doubt. Small decreases in the treated tissue were observed but the differences were not consistent. In the present experiment samples of dried tissue from several of the series of treatments were available for analysis and attention was paid only to the not-at-eye tissue. The results were consistent in showing lower starch values in the treated tissues, but the differences were small. Furthermore, although the optimum concentration of chemical showed the largest decreases in starch, the relation between the concentration of chemical and the decrease in starch was not such as to give clear gradations of values such as were obtained with the sucrose tests.

EXPERIMENTAL

Experimental tubers.—These were for the most part the same as those reported upon in the preceding article (3). In order to show the source of seed, time of harvesting, time of treatment, etc., table 1 has been prepared. These data apply to all of the lot numbers in the other tables in this paper.

TABLE 1. *Data on Tubers Used in Experiments*

| Lot No. | Variety | Source of Tubers | Date Harvested | Date Treated | Date Sampled |
|---------|---------|----------------------------|----------------|--------------|--------------|
| 137-141 | Cobbler | Maryland | * | July 25 | July 31 |
| 152-155 | " | " | * | Aug. 1 | Aug. 5 |
| 157-160 | " | " | * | " 3 | " 7 |
| 162-164 | Bliss | Yonkers, Nepperhan Gardens | Aug. 6 | " 8 | " 12 |
| 167-171 | " | " " " | " 6 | " 9 | " 14 |
| 177-181 | " | " " " | " 6 | " 13 | " 17 |
| 182-185 | " | " " " | " 6 | " 16 | " 20 |
| 187-190 | " | " " " | " 6 | " 19 | see table 3 |
| 207-210 | Cobbler | " " " | " 6 | " 26 | Aug. 31 |
| 211-212 | " | " " " | " 6 | " 26 | see table 3 |
| 213-214 | Bliss | " " " | " 6 | " 26 | " " " |
| 221-223 | Cobbler | " " " | " 6 | Sept. 3 | " " " |
| 224-227 | Bliss | Country Club Gardens | Sept. 3 | " 11 | Sept. 17 |
| 228-231 | Cobbler | " " " " | " 3 | " 11 | " 16 |
| 232-235 | Bliss | " " " " | " 3 | " 14 | " 19 |
| 236-239 | Cobbler | " " " " | " 3 | " 14 | " 19 |
| 240-243 | Bliss | " " " " | " 3 | " 12 | " 20 |
| 252-255 | Cobbler | " " " " | " 3 | " 18 | " 23 |

* About the middle of July, exact date not known.

Chemical treatments.—Three different chemicals were used: ethylene chlorhydrin ($\text{CICH}_2\text{CH}_2\text{OH}$), sodium thiocyanate (NaSCN), and thiourea (NH_2CSNH_2). Two different methods of treating with ethylene chlorhydrin were used: (1) Whole tubers were exposed for 24 hours to vapors of ethylene chlorhydrin in a closed container; the amounts of chlorhydrin (40 percent solution) used per liter of air space inside the container (without allowance for the volume of the tubers) ranged from 1.0 to 0.008 cc. (2) Seed-pieces obtained by cutting whole tubers into pieces about 25 grams each in weight were dipped into a dilute solution of ethylene chlorhydrin

and the dipped pieces were then placed in a container with a tight seal for a definite period, usually 24 hours, in some cases 16 hours, or even 8 hours; the concentration of the dipping solution ranged from 45 cc. to 5 cc. per liter of water.

The treatments with both sodium thiocyanate and thiourea consisted in soaking the cut tubers (not whole tubers) in the chemical solution for one hour and then planting without rinsing off the adhering chemical. The strength of the solution was varied from about one or two percent (10-20 grams per liter of water) to about one-fourth or one-eighth percent or even less. The exact amounts of chemicals used in each series are shown in column 2 of table 2.

In each series of concentrations used in each experiment the highest concentration is that which previous experience had shown to be approximately the optimum for these varieties and for this stage of dormancy. For the whole-tuber vapor treatments, one cc. per liter of air space for 24 hours is probably too high if the potatoes are to be cut and planted at once after treatment, but in the present experiments the treated tubers were not cut and planted at once after treatment but were allowed first to stand in air for five to seven days under which conditions this amount of chemical is not too high. For the thiourea treatments two percent is probably less effective than one percent for inducing good growth of sprouts after germination has started. Furthermore two percent thiourea induces the sprouting of too many sprouts per eye.

Sampling.—The methods of sampling the treated and check lots, and of squeezing the tissue to obtain press-juices are described in the previous article (2).

The time of sampling after treatment, as shown in the previous report (3), varied from four to seven days, and was chosen so as to allow the early stages in the initiation of germination to begin, but to avoid sampling when sprouting had taken place to any marked extent. In all cases, therefore, the samples were taken either before any sprouts were visible, or, in certain exceptional cases, when only a few of the seed-pieces in the treated lots showed evidence of the beginning of bud development.

Chemical methods.—For the sugar analyses a sample of the press-juice was dropped from a pipette into boiling 95 percent alcohol of sufficient volume to give a final concentration of 70 percent alcohol. When the analysis was started it was made up to volume in a flask and filtered. Aliquots of the alcoholic extract were placed in evaporating dishes, the alcohol removed by evaporation on a steam-bath and replaced gradually by water. The aqueous solution was made up to volume and aliquots taken for copper reduction before and after inversion. The inversion was carried out by acid in the cold (see 1, p. 95).

For the starch determinations samples of fresh tissue chopped to small pieces in a wooden bowl were dropped into boiling 95 percent alcohol and

allowed to boil for a few minutes. The dish was then placed on a steam-bath until nearly all of the alcohol was removed. The tissue was then placed before an electric fan and when it had become dry it was ground in a food-grinder and finally reduced to a fine powder in a power-driven grinder operating on the mortar-and-pestle principle. This powder was dried in an electric oven at 99° C. and portions weighed out for starch analysis. The acid-hydrolysis method (see 1, p. 95) was used, and the starch data, therefore, represent total acid-hydrolyzable substances calculated as starch. Some determinations were made with the Walton and Coe method (9) which eliminates the non-starch polysaccharids and thus gives a better figure for starch. But duplicate determinations on several samples of potato powder by the two methods showed that the Walton and Coe method gave a value uniformly about 0.9 of the acid-hydrolysis method indicating that non-starch polysaccharids formed only about one-tenth of the total formed by the acid-hydrolysis method. Because of the greater convenience of the acid method it was used exclusively in the rest of the tests.

Sprouting response.—The percentage sprouting for most of the lots is given in the preceding paper (3), p. 505. The percentages varied with different experiments, but a general statement is as follows: favorable concentrations of chemical induced 75 to 100 percent sprouting, the next lower concentration (which was about one-half to one-third of the optimum) induced 50 to 75 percent sprouting, while the values for the lower amounts of chemical were 20 to 50 percent; the check lots ranged from 0 to 20 percent. The important consideration is that the sprouting response formed a series corresponding to the concentrations of chemicals used in treating the potatoes.

RESULTS

Relation of Concentration of Chemical to the Development of Sucrose in the Press-juice of Potatoes

The sucrose contents of the juices obtained from the various lots which had been treated with different concentrations of chemicals are shown in table 2. In columns 2, 5, and 8 will be found the series of concentrations used in each test, the strength of the chemical being reduced step-wise, each lower concentration being some fraction, *e.g.*, one-half, one-fourth, one-third, etc., of the preceding value. In columns 3, 6, and 9 are shown the sucrose values in milligrams per five cc. of juice. It is seen that a gradation of sucrose values was obtained corresponding closely to the graded concentrations of chemicals used in treating the potatoes. Thus the favorable concentrations of ethylene chlorhydrin approximately doubled the sucrose content as compared with the check lot; the NaSCN-treated lots were about three times and the thiourea-treated lots were about 2.5 times as high in sucrose as the corresponding checks. When the concentration of chlorhydrin was reduced to one-third of the optimum value the

sucrose in the treated was then found to be about 1.5 times that of the check, and reducing the concentrations of NaSCN and thiourea to one-half strength caused the sucrose value to become about twice that of the check. Still further reductions in concentrations resulted in smaller differences between treated and checks, but even when the concentration was one-fourth or one-fifth the optimum gains of treated over checks were observed.

TABLE 2. *Effect of Chemical Treatment of Potatoes Upon the Sucrose in the Press-juice*

| Ethylene Chlorhydrin Treatments * | | | Sodium Thiocyanate Treatments | | | Thiourea Treatments | | |
|--------------------------------------|-------------------|----------------------------|----------------------------------|-------------------|----------------------------|------------------------|-------------------|----------------------------|
| Lot No. | Conc. of Chem. | Sucrose mg. in 5 cc. | Lot No. | Conc. of Chem. | Sucrose mg. in 5 cc. | Lot No. | Conc. of Chem. | Sucrose mg. in 5 cc. |
| 137 | 1.00 cc. | 54.0 | 157 | 2.00% | 31.2 | 152 | 2.00% | 39.8 |
| 138 | 0.20 " | 35.1 | 158 | 0.67% | 35.6 | 153 | 0.67% | 29.4 |
| 139 | 0.04 " | 26.2 | 159 | 0.22% | 22.4 | 154 | 0.22% | 22.4 |
| 140 | 0.008 " | 26.0 | 160 | check | 16.3 | 155 | check | 16.6 |
| 141 | check | 21.3 | | | | | | |
| 240 | 0.50 cc. | 56.2 | 167 | 1.00% | 55.7 | 162 | 2.00% | 57.3 |
| 241 | 0.17 " | 54.8 | 168 | 0.500% | 37.5 | 163 | 1.00% | 43.5 |
| 242 | 0.06 " | 42.4 | 169 | 0.250% | 27.4 | 165 | 0.50% | 40.1 |
| 243 | check | 38.2 | 170 | 0.125% | 14.1 | 166 | 0.25% | 34.6 |
| | | | 171 | check | 12.1 | 164 | check | 16.9 |
| 207 | 45 cc. | 23.8 | 182 | 1.00% | 60.0 | 177 | 1.000% | 57.9 |
| 208 | 15 " | 16.5 | 184 | 0.50% | 25.4 | 178 | 0.400% | 46.9 |
| 209 | 5 " | 11.9 | 186 | 0.25% | 25.2 | 179 | 0.160% | 42.8 |
| 210 | check | 13.3 | 183 | check | 19.5 | 180 | 0.064% | 37.1 |
| | | | 185 | check | 18.6 | 181 | check | 22.9 |
| 255 | 45 cc. | 25.9 | 235 | 1.00% | 71.1 | 224 | 1.00% | 43.7 |
| 254 | 15 " | 17.7 | 234 | 0.50% | 54.0 | 225 | 0.50% | 42.6 |
| 252 | 5 " | 17.7 | 233 | 0.25% | 41.1 | 226 | 0.25% | 39.0 |
| 253 | check | 13.3 | 232 | check | 17.7 | 227 | check | 23.4 |
| | | | 239 | 1.00% | 58.5 | | | |
| | | | 238 | 0.50% | 37.4 | | | |
| | | | 237 | 0.25% | 38.1 | | | |
| | | | 236 | check | 17.5 | | | |

* Lots 137-141 and 240-243 treated by the whole tuber method and column 2 shows the number of cubic centimeters of 40 percent ethylene chlorhydrin used per liter of air space inside the container. Time of exposure to vapors, 24 hrs. Lots 207-210 and 252-255 treated by the cut-tuber dip-method and column 2 shows the number of cubic centimeters of 40 percent ethylene chlorhydrin added to one liter of water in preparing the solution into which the cut-tubers were dipped before storing in a closed container for 24 hrs.

NOTE: For varieties, source, dates of harvest, etc., corresponding to the various lot numbers, see table 1.

Time Relation in the Sucrose Increase After Treatment

In this experiment samples of treated and check potatoes were removed at intervals of 24, 48, 72, 96, and 144 hours after treatment and the press-juices were analyzed for sugar. The results are shown in table 3, the time

TABLE 3. *Time Relation of Gain in Sucrose*

| Time After End of Treatment | Sucrose, Milligrams per 5 cc. of Press-juice | | | | | | | | | | |
|--------------------------------|---|-------|---|-------|---|-------|---|-------|----------------------------------|---------------------|-------|
| | Ethylene Chlorhydrin Treatment Lots. 211-212 * | | Ethylene Chlorhydrin Treatment Lots. 213-214 * | | Ethylene Chlorhydrin Treatment Lots. 187-188 † | | Sodium Thiocyanate Treatment Lots. 189-190 | | Lot Nos. 221, 222, and 223 | | |
| | Treated | Check | Treated | Check | Treated | Check | Treated | Check | Sodium Thiocyanate Treated | Thiourea Treated | Check |
| | | | | | | | | | | | |
| Start | — | 17.7 | | 32.1 | 24.0 | 19.8 | 26.3 | 29.6 | 13.6 | 14.4 | 11.6 |
| 24 hrs. | 6.9 | 11.0 | 17.7 | 19.6 | 15.7 | lost | 31.2 | 14.5 | 18.3 | 7.2 | 6.4 |
| 48 " | 16.6 | 7.8 | 27.2 | 16.0 | 16.8 | 14.1 | 34.4 | 17.4 | 21.2 | 19.1 | 13.0 |
| 72 " | 27.8 | 10.5 | 27.2 | 16.0 | 28.3 | 18.2 | 52.1 | 13.0 | 49.3 | 45.1 | 17.4 |
| 96 " | 48.4 | 17.7 | 35.3 | 16.8 | 25.2 | 15.9 | 54.1 | 13.6 | lost | 44.0 | 19.7 |
| 144 " | | | | | 52.9 | 22.4 | 50.0 | 16.9 | 38.5 | 38.5 | 10.7 |

* Dip method, dipping solution 60 cc. per l., storage period 16 hours. (Check lots dipped in H₂O.)† Dip method, dipping solution 30 cc. per l., storage period 24 hours. (Check lots dipped in H₂O.)

NOTE: Check lots 190 and 223 soaked in water instead of sodium thiocyanate or thiourea solution.

(after the end of the treatment) at which the samples were removed being shown in column 1, and the sucrose values of treated and check juices at each sampling period being shown side by side in paired columns under the appropriate heading for the type of treatment applied. Increases in sucrose of the treated over the check were observed with the sodium thiocyanate treatments at the end of the 24 hour period. In general, however, it was not until the 48th hour that the increase became pronounced and in the case of the chlorhydrin lot Nos. 187-188 the increase did not occur until sometime between the 48th and 72d hour. It will be noted that the check lots usually lost sucrose during the first 24 hour period as a result perhaps of the high rate of respiration induced by cutting the tuber into pieces. With the thiocyanate treated lots, however, this loss in sucrose was not noted, the early and rapid production of sucrose probably more than compensating for the excessive loss by respiration. It is likely that the increased respiration accounts for the low values at the 24 hour period in the chlorhydrin-treated lots, since as shown by Smith (7) the chlorhydrin treatment promptly induces a high rate of respiration.

The maximum increase was reached by the NaSCN and thiourea lots at about the 72d or 96th hour at which times the sucrose of the treated lots was about three times as great as in the corresponding checks. The chlorhydrin treatments did not reach their maximum difference until about the 96th or 144th hour, and showed at that time sucrose values about twice those of the checks.

Effect Upon the Reducing Sugars

Although in all cases shown in tables 2 and 3 the amount of reducing sugar was determined it seemed unnecessary to present the reducing sugar data in full for the reason that no relation was observed between the treatment that was applied and the amount of reducing sugar obtained. A few of the results which are characteristic of the whole are shown in table 4. The values for the check lots show that the amounts of reducing sugar were quite different in different lots of potatoes, there being in some cases 15 times as much reducing sugar as in others; consequently, so far as the relation of composition to dormancy is concerned, the reducing sugar may be either high or low in two lots and both lots may be dormant. But the important question in these experiments is what effect the treatments have had upon the amount of reducing sugar that is present, irrespective of whether this is high or low at the beginning of the treatment. By comparing the values in table 4 it is seen that in no case was a series of values obtained corresponding to the series of concentrations of chemicals used in treating the potatoes. In this respect, therefore, the behavior of the reducing sugar was in contrast to that of the cane sugar. This is in agreement with measurements reported in the previous paper (2) in which there was no evidence of a consistent effect of the treatments in either increasing or decreasing the amount of reducing sugar.

TABLE 4. *Effect of Chemical Treatment of Potatoes Upon Reducing Sugar in Press-juice*

| Ethylene Chlorhydrin Treatments | | Sodium Thiocyanate Treatments | | Thiourea Treatments | |
|---------------------------------|-----------------------------|-------------------------------|-----------------------------|---------------------|-----------------------------|
| Lot No. | Reducing Sugar in 5 cc. mg. | Lot No. | Reducing Sugar in 5 cc. mg. | Lot No. | Reducing Sugar in 5 cc. mg. |
| 207 | 1.5 | 157 | 18.6 | 152 | 18.6 |
| 208 | 1.8 | 158 | 30.6 | 153 | 17.4 |
| 209 | 2.1 | 159 | 30.0 | 154 | 14.8 |
| 210 | 1.2* | 160 | 23.1* | 155 | 17.4* |
| 255 | 1.4 | 235 | 17.7 | 224 | 15.1 |
| 254 | 1.5 | 234 | 18.3 | 225 | 7.2 |
| 252 | 2.3 | 233 | 18.4 | 226 | 13.8 |
| 253 | 1.5* | 232 | 19.5* | 227 | 3.9* |

* Check lot.

NOTE: For variety, source of tuber, time of digging corresponding to lot numbers, see table 1; and for concentrations of chemicals corresponding to lot numbers, see table 2.

Starch Changes

The starch changes are shown in table 5. The lot numbers are the same as those for the sucrose data but the starch determinations were made upon dried and powdered whole tissue and not upon the press-juices. The percentages of starch are shown in columns 3, 4, 8, 9, 13, and 14 in table 5, columns 3, 8, and 13 showing the duplicate determinations and columns 4, 9, and 14 showing the averages of the two duplicates in each case. Columns 5, 10, and 15 in table 5 show the percentage change of treated with respect to the corresponding check lot in each series. Thus lot 137 was 4.8 percent lower than the check lot 141, this value being calculated, not as percent of starch in the tissue, but as percent of the check value in each experiment.

It will be observed that the highest concentration of chemical in each experimental series showed the lowest percentages of starch; this is true for all three chemicals. The starch in the treated when calculated with respect to the corresponding check showed losses varying in different experiments from 3.0 percent in lot 207 to 10.6 percent in lot 228. Lower concentrations of chemical showed smaller differences between treated and check, the differences in some cases being small and probably negligible, *e.g.*, lot Nos. 208, 209, 170, 238, 237, 180, and 230; in fact, two of the treated lots in which low concentrations of chemical were used, lot Nos. 139 and 140, showed higher starch values than the check lot No. 141.

Since the differences in starch values were small the values for the duplicate determinations are given in each case in order to take into account the question whether the differences between treated and check lots are in excess of the error of the determination itself. There are available 27 pairs of duplicate determinations, each pair of measurements having been made with a different lot of potato-powder. Fleisch (4) has proposed a method for estimating the analytical error in such cases, and, applying his method

TABLE 5. *Effect of Chemical Treatment of Potatoes Upon the Starch Content of Tissue*

| Ethylene Chlorhydrin Treatments | | | | | Sodium Thiocyanate Treatments | | | | | Thiourea Treatments | | | | |
|---------------------------------|----------------|--------------|------|--|-------------------------------|----------------|--------------|------|--|---------------------|----------------|--------------|------|--|
| Lot No. | Conc. of Chem. | Starch * | | | Lot No. | Conc. of Chem. | Starch * | | | Lot No. | Conc. of Chem. | Starch * | | |
| | | % Dry Wt. | | Gain (+) or Loss (-) as % of the Check Value | | | % Dry Wt. | | Gain (+) or Loss (-) as % of the Check Value | | | % Dry Wt. | | Gain (+) or Loss (-) as % of the Check Value |
| | | Dupl. Det'ns | Ave. | | | | Dupl. Det'ns | Ave. | | | | Dupl. Det'ns | Ave. | |
| 137 | 1.00 cc. | 73.7 | 73.8 | -4.8 | 167 | 1.000% | 72.0 | 70.8 | -7.1 | 177 | 1.000% | 70.7 | 70.8 | - 5.6 |
| 138 | 0.20 " | 73.9 | | | 168 | 0.500% | 69.6 | | | 178 | 0.400% | 71.0 | | |
| | | 74.4 | 74.4 | -4.0 | | | 71.8 | 70.8 | -7.1 | | | 73.7 | 72.8 | - 2.9 |
| 139 | 0.04 " | 74.4 | | | 169 | 0.250% | 69.7 | | | 179 | 0.160% | 71.9 | | |
| | | 79.6 | 79.5 | +2.6 | | | 71.4 | 72.1 | -5.4 | | | 73.3 | 72.8 | - 2.9 |
| 140 | 0.008 " | 78.5 | | | 170 | 0.125% | 72.8 | | | 180 | 0.064% | 72.4 | | |
| | | 76.6 | 77.6 | +0.1 | | | 74.1 | 74.3 | -2.4 | | | 73.3 | 73.8 | - 1.6 |
| 141 | check | 78.6 | | | 171 | check | 74.5 | | | 181 | check | 74.4 | | |
| | | 77.6 | 77.5 | | | | 76.7 | 76.2 | | | | 74.0 | 75.0 | |
| | | 77.5 | | | | | 75.6 | | | | | 76.0 | | |
| 207 | 45 cc. | 75.4 | 75.0 | -3.0 | 239 | 1.00% | 68.7 | 68.5 | -3.8 | 228 | 1.00% | 62.5 | 63.2 | -10.6 |
| 208 | 15 " | 74.7 | | | 238 | 0.50% | 68.3 | | | 229 | 0.50% | 63.9 | | |
| | | 75.9 | 75.6 | -2.2 | | | 69.6 | 70.0 | -1.7 | | | 65.5 | 66.1 | - 6.5 |
| 209 | 5 " | 75.3 | | | 237 | 0.25% | 70.3 | | | 230 | 0.25% | 65.7 | | |
| | | 76.0 | 76.3 | -1.3 | | | 69.3 | 70.0 | -1.7 | | | 69.2 | 69.7 | - 1.4 |
| 210 | check | 76.6 | | | 236 | check | 70.7 | | | 231 | check | 70.1 | | |
| | | 77.1 | 77.3 | | | | 71.9 | 71.2 | | | | 71.1 | 70.7 | |
| | | 77.6 | | | | | 70.6 | | | | | 70.3 | | |

* By acid-hydrolysis method, gain in reducing substances calculated as starch.

NOTE: For description of lot numbers, see table 1. For description of treatments for lots 137-141 and 207-210, see table 2.

to the present case, it is found that the difference between two pairs of duplicates should be about two percent (*i.e.* two percent of the amount of starch found) to be considered significant. All of the differences shown by the highest concentrations of chemical are found to be greater than this value, and also some of those shown by lower concentrations.

We may consider the matter in another way, namely, by comparing the differences between check and treated lots of tissue with the average difference between two duplicate determinations of a single lot of tissue. We find that the average deviation of a single starch measurement from the mean of the two duplicates is about 0.7 percent of the average starch value of the two; on this basis the starch differences shown by the highest concentrations of chemical and the corresponding checks are at least four times and as much as 15 times the average deviation of duplicates; for the next lower concentration the treated lots range from two to ten times the average error; but for the next lower concentration three of the six lots do not show ratios greater than twice the error.

My conclusion is that the higher concentrations of chemical have caused decreases in the starch content as compared with the check lots, but that when the concentration was reduced to about one-third of the optimum concentration the differences between treated and checks become of doubtful significance. And although a general relation was found between the concentration of chemical and starch content, in that the higher concentrations have given low starch values, and *vice versa*, the gradations have not been such as to give a series of values corresponding closely to the series of concentrations of chemical used in the treatments, as was the case for the sucrose.

DISCUSSION

It is interesting that the relation of the sucrose to the chemical treatments is such as to give a series of values corresponding so closely to the concentrations of chemicals used in the treatments. Tests made on press-juice from potatoes which had been soaked one hour in one percent sodium thiocyanate indicated an absorption of sufficient sodium thiocyanate to give only about 0.01 gram NaSCN per 100 cc. of press-juice; the amount of ethylene chlorhydrin vapor taken up by the tissue in the treatments has not been determined, but even in the cases of the highest concentrations used it must have been relatively small as compared with the NaSCN values. These facts show how responsive the cells of the tuber are to comparatively small changes in concentration of chemicals.

The gradations that have been observed may be related to gradations in dormancy of different eyes in the same lot. That is, perhaps the lower concentrations were strong enough to start the activity of only the least dormant eyes, while the stronger concentrations could break the rest period of those in the most deeply dormant condition.

The difference in the behavior of the reducing sugar and the sucrose

should be emphasized. There is a tendency in the literature to class these two groups together as "sugar," and yet in these experiments there has been a qualitative difference between the response of the two, sucrose showing increases in a perfectly definite manner and the reducing sugar showing an irregular behavior.

It is not suggested that the breaking of dormancy is caused by the sucrose accumulation, and that growth starts when the sucrose content increases to a sufficient value. Soaking potatoes in a sucrose solution, or injecting it into the tissue even near the eye of the potato, does not induce growth of buds in dormant tubers. It seems more likely that the sucrose increase is mainly a result and not a cause. At present we can regard the sucrose increase only as evidence that the chemical treatments have become effective in the tissues of the tuber, and that subsequently sprouting of the buds will become evident.

These results which indicate an increase in sucrose following a decrease in starch, are of special interest in connection with the views of certain authors that there is some sort of a direct connection between starch and sucrose. This would not be expected in view of the fact that when starch is broken down by enzymes which can be separated from plant tissue maltose, not sucrose, is obtained. But there are several suggestions in the literature that a starch-sucrose equilibrium exists, and that sucrose is an intermediate product between starch and dextrose. The reader is referred to papers by Kayser (5), Ripperton (6), de Wolff (10), and Tollenaar (8) for further information on this point. It has often been found that as starch decreases sucrose increases; but it is merely an assumption to say that the connection is direct; it is better to say that we do not know what the steps are by which we may connect the disappearance of starch with the appearance of sucrose.

Although both the previous and present reports show that, in the treated lots as compared with the checks, starch decreased and sucrose increased, it should not be inferred that the changes in absolute amounts of these substances in the tissue were large. The actual changes were, in fact, relatively small. Thus, in the present experiments, assuming the moisture content of the tissue as 80 percent, and assuming that 80 cc. of press-juice are equivalent to 100 grams of fresh tissue, the excess loss of starch from a 25 gram treated seed-piece as compared with a check seed-piece was about 0.1 to 0.4 gram, and the gain in sucrose was about 0.05 to 0.2 gram. We may also recalculate the changes shown by the data from the previous report (2, table 2, p. 331) from which we find that the starch loss per 25 gram seed-piece was about 0.2 gram and the sucrose increase about 0.1 gram greater in the treated than in the check. These are relatively small absolute changes in material. Furthermore, these are the changes induced by favorable concentrations of chemical, and the data show that much lower concentrations have an observable effect not only upon the

sucrose and starch changes, but also upon the sprouting response. These facts show that the transition from dormancy to growth can take place without involving any extensive change in the total amounts of materials present in the tissue.

SUMMARY

1. Freshly harvested potatoes (*Solanum tuberosum* L.) were treated with ethylene chlorhydrin, sodium thiocyanate, and thiourea, the concentrations of the chemicals being decreased from the optimum by steps to form a graded series. Press-juices from the treated potatoes obtained from the various lots at a subsequent interval, usually four to seven days before sprouting became visible, were compared with juices from the checks with reference to sugar content.

2. Sucrose was found to be higher in the treated than in the check lots, and, furthermore, to form a graded series of values corresponding to the series of concentrations of chemicals used in treating the potatoes.

3. The reducing sugar values did not form such a series, and no consistent effect of the treatments in either increasing or decreasing the reducing sugar content was found.

4. When samples were taken at intervals of 24, 48, 72, etc., hours after treatment it was found that the time after treatment at which the sucrose content of the treated lots became higher than that of the checks differed in different experiments, being as early as 24 hours and as late as 72 hours.

5. Samples of entire tissue which had been dried, powdered, and analyzed for starch showed that concentrations of chemical favorable for breaking the dormancy of the sprouts caused decreases in the starch; with low concentrations of chemical, however, the differences were small and of doubtful significance.

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THE TWIN-LEAF METHOD OF STUDYING CHANGES IN LEAVES¹

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INTRODUCTION

When measuring the changes which take place in leaves at intervals during a day by removing a sample of leaves at the beginning of the experimental period, and taking a second sample at the end of the period for comparison with the first one, it is important that the two samples be strictly comparable. If the differences between the two lots are to be taken as a measure of the change during the interval, we must be certain, first of all, that they were equal at the start.

So great is the variation in leaves upon a plant that to obtain two samples containing leaves of the same age, weight, and chemical composition by making a general collection requires a large number of leaves in order that these individual variations may be equalized. For many types of experiments this requirement can not be fulfilled because of the limitations set by space, by the numbers of plants available, by the details of technique involved in setting up the experiment, etc. If it is necessary to use relatively small numbers of plants, how shall we arrange to obtain the required number of comparable samples so that a series of samples over a considerable period of time may be obtained?

Sachs (9) offered a solution of this problem by proposing the use of what has now come to be known as the "half-leaf" method. He cut the leaf in two, lengthwise along the midrib, taking the first half as a sample to represent the condition at the beginning, and leaving the other half upon the plant for removal at the end of the desired period. Usually the entire half leaf was not used but a measured area was cut out using a template, and the results of the determination were expressed on the leaf area basis.

In later years this method was subjected to criticisms; first, that the opposite halves of the leaf were in fact not symmetrical, and further, that on account of fluctuations in area due to shrinkage when water was lost and to distention when water was gained, the computations on the leaf area basis were erroneous. Thoday (12) made these criticisms the object of a special investigation and found that both sources of error were important

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factors, that the asymmetry error was inherent in the method and could not be avoided, and that only the shrinkage error could be overcome. He suggested (13) an improvement which consisted in marking out at the beginning with a rubber stamp the area that was to be taken at the end of the period, thus nullifying the effect of any change in area that might occur during the experimental period.

Recently two more objections to the Sachs method have been made, these referring to errors arising from mutilation of the leaf in cutting away the half leaf or portion of it. Combes (4) claims that the wounding of the leaf in cutting increases the rapidity of translocation from the portion that remains, the effect being so great that he could distinguish between different degrees of wounding by the effect on translocation; and von Guttenberg (7) reports that removing a half leaf induces even qualitative changes in the composition of the remaining half; that, for example, in *Ilex* the wounding causes the appearance of saccharose in the other half leaf at a time when this sugar does not normally exist in it, and that a similar condition prevails as to glucosides in *Hedera*. He speaks of the method as leading to pathological conditions in the leaf.

There seems to be a need, therefore, for an alternative method that can avoid some of these difficulties of the half-leaf method. The present experiments were started for the purpose of testing the possibility of using the paired leaves of plants with opposite leaves or of leaflets of compound leaves, the plan in general being to take one leaf of the pair at the beginning of the experimental period, and the opposite one at the end. These opposite leaves are of exactly the same age, and, in some species at least, are so nearly the same in shape, size, and composition that we may regard them as twins. For this reason it is suggested that the method be called the "twin-leaf" method to distinguish it from the "half-leaf" method. And if it should be found from later more extensive tests that leaf pairs in general are not sufficiently alike to justify the word "twin" then the term "opposite-leaf" could be used.

Chibnall (3) used the opposite leaflets of *Phaseolus* in his study of the diurnal changes in nitrogen distribution, and Curtis (5) applied this method in measuring the effect of the cooling of petioles upon the rate of translocation from leaf blades. But both Chibnall and Curtis were interested in the use of the method for the purposes of their particular problems and not in the method itself; consequently they do not show in detail what the variation is between opposite leaves or leaflets, or make any effort to extend the application of the method to species other than the ones they used. It has been the purpose of the present experiments to explore the field in this direction, to measure the amount of variation likely to be encountered in paired leaves, to note by means of chemical analysis to what extent opposite leaves have the same composition, and finally to apply the method to the problem of determining the changes in leaves at intervals during a 24 hour period.

AMOUNT OF VARIATION IN OPPOSITE LEAVES

In order to determine the variation in weight of opposite leaves of various species, samples of paired leaves of the species shown in table 1 were taken. In obtaining the samples the two leaves of each pair were

TABLE 1. *Variations in Fresh Weights of Opposite Leaves or Leaflets*

| Species | Fresh Weights of Opposite Leaves,† Grams | | % Total Dev. |
|---|---|--------|--------------|
| | a. | b. | |
| <i>Coleus Blumei</i> Benth. (yellow)..... | 13.913 | 14.341 | 3.1 |
| <i>Coleus Blumei</i> Benth. (varieg.)..... | 7.424 | 7.374 | 0.7 |
| <i>Glycine Max</i> Merr..... | 8.078 | 8.023 | 0.7 |
| <i>Glycine Max</i> Merr..... | 8.013 | 8.052 | 0.5 |
| <i>Rosa rugosa</i> Thunb..... | 5.132 | 5.117 | 0.3 |
| <i>Rosa rugosa</i> Thunb..... | 4.944 | 4.923 | 0.4 |
| <i>Ailanthus altissima</i> Swingle..... | 5.496 | 5.554 | 1.1 |
| <i>Ailanthus altissima</i> Swingle..... | 7.678 | 8.013 | 4.2 |
| <i>Gardenia jasminoides</i> Ellis..... | 11.660 | 11.209 | 3.9 |
| <i>Bryophyllum calycinum</i> Salisb..... | 39.857 | 40.482 | 1.8 |
| <i>Syringa vulgaris</i> L..... | 5.120 | 5.274 | 3.0 |
| <i>Helianthus debilis</i> Nutt.*..... | 29.504 | 29.355 | 0.5 |
| <i>Lonicera Standishii</i> Carr..... | 4.059 | 4.213 | 3.8 |
| <i>Melilotus alba</i> Desr..... | 1.422 | 1.453 | 2.2 |
| <i>Melilotus alba</i> Desr..... | 1.245 | 1.225 | 1.6 |
| <i>Deutzia gracilis</i> Sieb. and Zucc..... | 5.524 | 5.305 | 4.0 |
| <i>Ligustrum ovalifolium</i> Hassk..... | 4.784 | 4.738 | 1.0 |
| <i>Ligustrum ovalifolium</i> Hassk..... | 4.043 | 4.088 | 1.1 |

* Cultivated form of sunflower, listed in the seed catalog as belonging to the cucumerifolius type. This form has opposite leaves only in the early stages of growth.

† In all cases both samples collected at the same time.

NOTE: 25 pairs of leaves or leaflets in each case except for *Melilotus* in which case 50 pairs of leaflets were used.

picked simultaneously, and were put in separate weighing bottles; this was repeated until 25 pairs of leaves were collected; these were then weighed and in table 1, the second column (under *a*) shows the total fresh weight of 25 leaves or leaflets, and the third column (under *b*) shows the total weight of the opposite organs. In column 4 will be found the percentage total deviation between the two samples, *i.e.*, the percent error that results from the assumption that the opposite leaves of a composite sample of 25 pairs of leaves are identical in weight. This value ranges in the experiment from 0.3 percent with *Rosa rugosa* to 4.2 percent with *Ailanthus jasminoides*. We can obtain an estimate of the amount of the error that would likely result if composite samples of 16, 9, or 4 leaves instead of 25 were taken by making use of the general relation that the error is inversely proportional to the square root of the number. The error for 16 leaves would then be about five-fourths, of nine leaves about five-thirds, etc., of the values shown in column 4 in table 1.

TABLE 2. Variation in Weights of Opposite Leaves of Pairs

| Leaves of Pair | <i>Salvia splendens</i> Ker. | | | | | | | | | | | | <i>Ligustrum ovalifolium</i> Hassk. | | <i>Helianthus debilis</i> Nutt. | |
|-----------------|------------------------------|---------|------------|---------|--------------------------|---------|------------|---------|--------------|---------|------------|---------|-------------------------------------|---------|---------------------------------|---------|
| | Tip Leaves | | | | Pair of Leaves Below Tip | | | | Third Pair | | | | | | | |
| | Fresh Wt. g. | % dev.* | Dry Wt. g. | % dev.* | Fresh Wt. g. | % dev.* | Dry Wt. g. | % dev.* | Fresh Wt. g. | % dev.* | Dry Wt. g. | % dev.* | Fresh Wt. g. | % dev.* | Fresh Wt. g. | % dev.* |
| <i>a</i> | 0.126 | 4.8 | 0.019 | 0.0 | 0.353 | 8.0 | 0.044 | 4.5 | 0.657 | 1.5 | 0.094 | 1.0 | 0.196 | 2.0 | 0.943 | 1.6 |
| <i>b</i> | 0.132 | | 0.019 | | 0.325 | | 0.042 | | 0.647 | | 0.095 | | 0.192 | | 0.928 | |
| <i>a</i> | 0.144 | 9.1 | 0.021 | 9.5 | 0.361 | 4.1 | 0.051 | 3.9 | 0.557 | 10.0 | 0.080 | 8.8 | 0.253 | 1.6 | 1.356 | 3.8 |
| <i>b</i> | 0.157 | | 0.023 | | 0.348 | | 0.049 | | 0.501 | | 0.073 | | 0.257 | | 1.408 | |
| <i>a</i> | 0.197 | 4.6 | 0.023 | 13.0 | 0.318 | 7.2 | 0.045 | 2.2 | 0.551 | 4.3 | 0.066 | 9.1 | 0.179 | 3.3 | 0.784 | 2.1 |
| <i>b</i> | 0.206 | | 0.026 | | 0.341 | | 0.046 | | 0.575 | | 0.072 | | 0.185 | | 0.768 | |
| <i>a</i> | 0.165 | 1.2 | 0.026 | 7.7 | 0.338 | 2.4 | 0.047 | 4.2 | 0.520 | 3.7 | 0.077 | 5.2 | 0.231 | 6.9 | 0.803 | 9.3 |
| <i>b</i> | 0.163 | | 0.024 | | 0.330 | | 0.045 | | 0.539 | | 0.081 | | 0.215 | | 0.728 | |
| <i>a</i> | 0.137 | 2.2 | 0.021 | 0.0 | 0.445 | 0.2 | 0.063 | 3.1 | 0.593 | 0.3 | 0.069 | 4.3 | 0.179 | 0.0 | 0.919 | 1.5 |
| <i>b</i> | 0.134 | | 0.021 | | 0.444 | | 0.065 | | 0.591 | | 0.072 | | 0.179 | | 0.933 | |
| <i>a</i> | 0.162 | 4.3 | 0.026 | 3.8 | 0.306 | 1.0 | 0.047 | 8.5 | 0.848 | 4.4 | 0.117 | 4.3 | 0.204 | 1.0 | 0.898 | 1.4 |
| <i>b</i> | 0.169 | | 0.027 | | 0.303 | | 0.043 | | 0.811 | | 0.112 | | 0.206 | | 0.910 | |
| <i>a</i> | 0.146 | 2.0 | 0.025 | 0.0 | 0.278 | 7.5 | 0.038 | 7.9 | 0.647 | 4.6 | 0.104 | 1.0 | 0.232 | 9.9 | 1.074 | 6.9 |
| <i>b</i> | 0.143 | | 0.025 | | 0.299 | | 0.041 | | 0.677 | | 0.103 | | 0.255 | | 1.148 | |
| <i>a</i> | 0.230 | 3.9 | 0.029 | 3.4 | 0.408 | 2.5 | 0.055 | 1.8 | 0.724 | 1.7 | 0.093 | 3.2 | 0.190 | 13.6 | 0.924 | 7.0 |
| <i>b</i> | 0.221 | | 0.028 | | 0.418 | | 0.054 | | 0.712 | | 0.090 | | 0.174 | | 0.860 | |
| <i>a</i> | 0.176 | 1.1 | 0.027 | 0.0 | 0.415 | 2.9 | 0.059 | 1.7 | 0.548 | 4.0 | 0.071 | 1.4 | 0.157 | 12.8 | 1.071 | 9.6 |
| <i>b</i> | 0.178 | | 0.027 | | 0.427 | | 0.060 | | 0.526 | | 0.070 | | 0.177 | | 0.968 | |
| <i>a</i> | 0.147 | 8.1 | 0.019 | 5.3 | 0.353 | 8.5 | 0.047 | 8.5 | 0.530 | 3.9 | 0.064 | 7.8 | 0.275 | 8.0 | 0.830 | 1.7 |
| <i>b</i> | 0.159 | | 0.020 | | 0.383 | | 0.051 | | 0.551 | | 0.069 | | 0.253 | | 0.844 | |
| Ave. % dev. 4.1 | | | | 4.3 | | 4.4 | | 4.6 | | 3.8 | | 4.6 | | 5.9 | | 4.5 |

* Percent deviation is difference between *a* and *b* expressed as percent of *a*.NOTES: In each case *a* refers to one leaf and *b* to the opposite leaf of this pair, the leaves being taken simultaneously.

The comparison was carried out in greater detail with leaves of *Salvia*, *Ligustrum*, and *Helianthus* as shown in table 2, in which will be found the weights of individual leaves. Thus, the two leaves at the tip of *Salvia* were weighed separately as shown in column 2 opposite *a* and *b*, respectively; then follow in column 4 the dry weights. The data for ten pairs of leaves from the tips of *Salvia* plants are thus shown in columns 2, 3, 4, and 5; and in columns 6, 7, 8, and 9 are shown the results of similar measurements for the paired leaves below the tip, etc. Also in the right hand columns of table 2 are shown the measurements with *Ligustrum* and *Helianthus*.

The percentage deviations of one leaf from the opposite leaf are shown in columns 3, 5, 7, 9, 11, 13, 15, and 17, table 2. These values are similar for the different types of leaves and are about four to six percent. This is the variation per single pair of leaves. If the sample was taken by combining 9, 16, or 25 pairs of such leaves the error would be reduced to approximately one-third, one-fourth, and one-fifth these values, respectively.

We may compare these values (which are errors due to lack of symmetry between opposite leaves) with the values obtained for asymmetry between opposite halves of the same leaf. Thoday (12) made measurements of the deviation of the weight of one half leaf from that of the other half, and found results which varied with different species but was commonly about 1.5 to 3.0 percent and sometimes more than 4.0 percent. Gouwentak (6) in a recent report on a study of diurnal changes in the nitrogen contents of *Helianthus* leaves gives (6, p. 44) the dry weights of opposite halves of leaves. There were eight leaves in the comparison, and while Gouwentak expresses the data on the grams-per-square-decimeter basis, we may calculate the values in the manner that was done for the opposite leaves of *Salvia*, i.e., by expressing the total deviation between the two halves as a percentage of the value for the leaf half marked *a* in Gouwentak's list. This gives the average percentage deviation between leaf halves as 3.2 percent. The values found by Thoday and by Gouwentak for the half-leaf method are, therefore, somewhat lower than the asymmetry errors of the twin-leaf method, as shown by tables 1 and 2 above, but the difference is not large, and considering the small numbers involved it can not be asserted that even this difference is significant. The twin-leaf method gives sufficiently low error values to warrant its consideration as a method of getting comparable samples of leaves.

VARIATION IN CHEMICAL COMPOSITION OF SAMPLES OF OPPOSITE LEAVES

Table 3 shows the differences in chemical composition of samples of opposite leaves, the differences in these cases representing not only errors in the sampling but also errors in the analyses. The *A* series was carried out in 1929 with composite samples of 30 leaves and the *B* series in 1930

TABLE 3. Chemical Composition of Samples of Opposite Leaves of *Salvia*. Series A in April 1929

| | Experiment No. 1 | | | | Experiment No. 2 | | | | Experiment No. 3 | | | | Experiment No. 4 | | | | Experiment No. 5 | | | |
|----------------|----------------------------|--------|--------------------|------|----------------------------|--------|--------------------|------|----------------------------|--------|--------------------|------|----------------------------|--------|--------------------|------|----------------------------|--------|--------------------|------|
| | Total in 30 Leaves (grams) | | % of the Fresh Wt. | | Total in 30 Leaves (grams) | | % of the Fresh Wt. | | Total in 30 Leaves (grams) | | % of the Fresh Wt. | | Total in 30 Leaves (grams) | | % of the Fresh Wt. | | Total in 30 Leaves (grams) | | % of the Fresh Wt. | |
| | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b |
| Fresh Wt.... | 18.432 | 18.266 | | | 12.006 | 12.058 | | | 12.428 | 12.299 | | | 10.249 | 10.583 | | | 11.779 | 11.749 | | |
| Dry Wt.... | 2.433 | 2.413 | 13.2 | 13.2 | 1.651 | 1.639 | 13.8 | 13.6 | 1.706 | 1.695 | 13.7 | 13.8 | 1.540 | 1.611 | 15.0 | 15.2 | 1.602 | 1.600 | 13.6 | 13.6 |
| Water..... | 15.999 | 15.853 | 86.8 | 86.8 | 10.355 | 10.419 | 86.2 | 86.4 | 10.722 | 10.604 | 86.3 | 86.2 | 8.709 | 8.972 | 85.0 | 84.8 | 10.177 | 10.149 | 86.4 | 86.4 |
| Insol. Solids. | 1.604 | 1.602 | 8.6 | 8.7 | 1.380 | 1.367 | 11.5 | 11.3 | 1.446 | 1.428 | 11.7 | 11.6 | 1.257 | 1.338 | 12.3 | 12.8 | 1.312 | 1.321 | 11.2 | 11.3 |
| Sol. Solids... | 0.829 | 0.811 | 4.5 | 4.4 | 0.271 | 0.272 | 2.6 | 2.3 | 0.260 | 0.267 | 2.2 | 2.2 | 0.283 | 0.273 | 2.8 | 2.6 | 0.290 | 0.279 | 2.5 | 2.4 |
| Starch..... | 0.560 | 0.541 | 3.04 | 2.98 | 0.555 | 0.564 | 4.62 | 4.65 | 0.644 | 0.652 | 5.18 | 5.30 | 0.617 | 0.633 | 6.06 | 6.03 | 0.610 | 0.603 | 5.22 | 5.15 |
| Insol. N.... | 0.108 | 0.112 | 0.59 | 0.62 | 0.061 | 0.058 | 0.51 | 0.48 | 0.052 | 0.054 | 0.42 | 0.44 | 0.043 | 0.046 | 0.42 | 0.44 | 0.045 | 0.048 | 0.39 | 0.41 |

Series B in May 1930

| | Experiment 6 Tip Leaves | | | | Experiment 7 Leaves Below Tip | | | | Experiment 8 Third Pair of Leaves | | | | Experiment 9 Tip Leaves | | | | Experiment 10 Leaves Below Tip | | | | Experiment 11 Third Pair of Leaves | | | |
|--------------|----------------------------|-------|--------------------|------|----------------------------------|-------|--------------------|------|--------------------------------------|-------|--------------------|------|----------------------------|-------|--------------------|------|-----------------------------------|-------|--------------------|------|---------------------------------------|-------|--------------------|------|
| | Total in 15 Leaves (grams) | | % of the Fresh Wt. | | Total in 10 Leaves (grams) | | % of the Fresh Wt. | | Total in 10 Leaves (grams) | | % of the Fresh Wt. | | Total in 15 Leaves (grams) | | % of the Fresh Wt. | | Total in 10 Leaves (grams) | | % of the Fresh Wt. | | Total in 10 Leaves (grams) | | % of the Fresh Wt. | |
| | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b | a | b |
| Fresh Wt.... | 2.631 | 2.614 | | | 3.710 | 3.639 | | | 6.041 | 6.129 | | | 1.630 | 1.662 | | | 3.575 | 3.618 | | | 6.175 | 6.130 | | |
| Dry Wt.... | 0.384 | 0.383 | 14.2 | 14.7 | 0.522 | 0.507 | 14.1 | 13.9 | 0.822 | 0.839 | 13.5 | 13.7 | 0.236 | 0.240 | 14.5 | 14.4 | 0.452 | 0.454 | 17.5 | 17.3 | 0.835 | 0.837 | 13.5 | 13.7 |
| Starch..... | 0.025 | 0.027 | 0.95 | 1.03 | 0.034 | 0.035 | 0.92 | 0.96 | 0.052 | 0.051 | 0.86 | 0.83 | 0.023 | 0.023 | 1.41 | 1.38 | 0.047 | 0.042 | 1.31 | 1.16 | 0.073 | 0.076 | 1.18 | 1.24 |
| Red. Sugar | 6.0* | 6.3* | 0.23 | 0.24 | 6.2* | 6.3* | 0.17 | 0.18 | 8.7* | 9.5* | 0.14 | 0.16 | 2.9* | 3.0* | 0.18 | 0.18 | 5.9* | 5.9* | 0.16 | 0.16 | 7.7* | 7.2* | 0.12 | 0.12 |
| Sucrose.... | 1.8* | 1.3* | 0.07 | 0.05 | 5.8* | 4.8* | 0.16 | 0.13 | 9.9* | 5.3* | 0.16 | 0.09 | 2.1* | 3.4* | 0.13 | 0.20 | 2.1* | 2.9* | 0.06 | 0.08 | 4.1* | 5.4* | 0.07 | 0.09 |
| Insol. N.... | 0.015 | 0.016 | 0.58 | 0.61 | 0.021 | 0.021 | 0.57 | 0.57 | 0.031 | 0.029 | 0.52 | 0.47 | 0.010 | 0.010 | 0.61 | 0.60 | 0.020 | 0.019 | 0.54 | 0.52 | 0.031 | 0.030 | 0.50 | 0.49 |

* Milligrams instead of grams.

NOTE: Leaves in experiments 9, 10, and 11 dried in an electric oven at 99° C.

NOTE: Starch determined by acid-hydrolysis method (see I, p. 95). NOTE: In each experiment both samples collected at the same time.

with composites of 10 to 15 leaves. It is seen that good agreement was obtained with constituents which represented fairly large weights of material per sample such as fresh weight, dry weight, and starch, but that with sugars the percentage error was large, due to the small amounts of these substances in the tissue.

METHODS OF ANALYSIS

For the experiments in April, 1929, the leaves (with petioles removed), after being weighed, were cut into pieces and dropped into boiling alcohol of sufficient volume to give a final concentration of 80 percent alcohol. When the process of extraction was started the liquid was decanted through a tared Soxhlet extraction thimble and finally all particles of tissue were transferred to it; the tissue was then extracted with alcohol in the Soxhlet apparatus and the extracts made up to volume. The residue in the thimble was dried in an oven at 99° C.; the difference in weight in comparison with the tared thimble gave the weight of insoluble solids; an aliquot of the extract was taken for the determination of the soluble solids; the sum of the insoluble solids and the soluble solids gave the dry weight, and the dry weight and fresh weight difference gave the water content. Samples from the residue which was insoluble under the conditions of the extraction were taken for insoluble nitrogen by the Kjeldahl method (1, p. 7), and also for the starch determination. This was carried out by the acid-hydrolysis method (1, p. 95) except in one case in which the Walton and Coe method (14) was used.

In the May, 1930, experiments the samples after being dropped into boiling alcohol were put into a weighed porcelain dish on a steam bath until the alcohol was evaporated; they were then dried in a vacuum oven at 70° C. The difference in weight gave dry weight for the leaves representing the second pair below the tip, but this value could not be obtained for the tip leaves and for the leaves representing the first pair below the tip in this experiment, since calcium carbonate had been added to the alcohol at the time the leaves were dropped into it. The dried residue was then transferred first to a mortar and ground up with 70 percent alcohol, and then to pyrex centrifuge tubes. After the liquid had been brought to boiling in the 70 percent alcohol and then allowed to cool, it was centrifuged and the extract was decanted; another quantity of 70 percent alcohol was added and a second extraction was made; in this way six extractions were carried out. The extracts were combined and aliquots were used for the sugar and for soluble nitrogen determinations. The entire residue was used for both starch (or more accurately acid-hydrolyzable polysaccharids) and insoluble nitrogen in the following manner: After the period of acid-hydrolysis the liquid was removed by successively centrifuging and decanting; the liquids were collected, neutralized, made up to volume, and five cc. samples taken for the Somogyi (11) modification of the Shaffer and Hartman micromethod (10); the residue and the liquid portion were digested

separately in Kjeldahl flasks; the digest-liquid from the residue was made up to volume and an aliquot of this exactly equal to the aliquot of the liquid portion was taken; these two digests were then recombined and represented a given aliquot of the original material; a Kjeldahl distillation then gave the amount of nitrogen in the insoluble portion of the sample of leaves.

DIURNAL CHANGES IN LEAVES

General Procedure

Salvia splendens Ker. was chosen for this experiment in which the object was to make use of the twin-leaf method for studying the changes in leaves at intervals of 2.5 to 4.0 hours throughout a 24 hour period. The plants were grown from seed, were about six to eight inches high, and had produced several pairs of leaves. When the plants are young the opposite leaves of *Salvia* are very uniform, but when they get older and when branching begins the leaves become coarser and less symmetrical.

Three types of leaves were sampled, the young tip leaves, the leaves of the pair below the tip, and the third pair of leaves; these represent the very young, the partly grown, and nearly full grown stages of leaves. The samples of the three types were kept separate, and one of the interesting results of the experiment has been to note the difference in the behavior of the three types.

The first experiment for diurnal changes was begun on April 3, 1929, samples being taken at 5:30, 8:00, 10:30 A.M., 1:00, 3:30, 7:00, 11:00 P.M. and 4:00 A.M. April 3 was a bright and sunny day. Thirty leaves were taken for each sample and the procedure in getting a series of comparable leaves throughout the day was as follows: at 5:30 A.M. one leaf of each of 30 pairs of opposite leaves was taken and the other 30 leaves were left until 8:00 A.M., at which time this second sample was collected; but at the same time another sample of 30 leaves from 30 other plants was taken, these representing the first sample for the period 8:00 A.M. to 10:30 A.M., the opposite leaves being left on for the sample at the end of the period. In this way samples at the beginning and end of each period were available from twin-leaves, and the change during each period could be deduced from the differences in these pairs, and expressed in absolute amounts of material, *i.e.*, grams of water, dry weight, starch, sugar, etc., or as the percentage of the fresh weight if this was considered advisable, since the data for this computation were available. In collecting each sample of leaves an effort was made to obtain leaves with varying positions toward the sun in order to equalize variations in this respect.

Another test was made May 12, 1930, sampling starting at 6:00 A.M. and continuing until 5:00 A.M., May 13. The method of sampling was the same as described for April 1929 except that a smaller number of leaves per sample was used, 20 of the tip-leaves and of the pair below the tip,

and 15 of the third pair of leaves. May 12 was entirely clear throughout the day.

Results of the April 1929 Experiment

The data for the April 1929 test are given in tables 4, 5, and 6 which show the total number of grams of material in each sample, the percentage change of each constituent, and the total amount at any period calculated with reference to the amount present at the start in the early morning. Table 4 shows the data for the tip-leaves, table 5 for the leaves just below the tip, and table 6 for the third pair of leaves (second below the tip).

TABLE 4. *Diurnal Changes in Composition of Salvia Leaves. Tip Leaves. April 1929 Experiment*

| Time | Total Amount in 30 Leaves | | | Percent Gain (+) or Loss (-) During the Period * | | | Relative Amount Present. Amount at 5:30 A.M. as 100 | | |
|---------------------------|---------------------------|----------------|----------------|--|---------|--------|---|---------|-------|
| | Fresh Wt. grams | Dry Wt. grams | Water grams | Fresh Wt. | Dry Wt. | Water | Fresh Wt. | Dry Wt. | Water |
| { 5:30 A.M. 8:00 A.M. | 7.164 7.346 | 0.898 0.952 | 6.266 6.394 | + 2.5 | + 6.0 | + 2.0 | 103 | 106 | 102 |
| { 8:00 A.M. 10:30 A.M. | 6.963 7.161 | 0.947 1.055 | 6.016 6.106 | + 2.8 | + 11.4 | + 1.5 | 105 | 118 | 104 |
| { 10:30 A.M. 1:00 P.M. | 6.702 7.179 | 1.062 1.159 | 5.640 6.020 | + 7.1 | + 9.1 | + 6.8 | 113 | 129 | 111 |
| { 1:00 P.M. 3:30 P.M. | 6.425 7.130 | 1.075 1.185 | 5.350 5.945 | + 11.0 | + 10.2 | + 11.3 | 125 | 142 | 123 |
| { 3:30 P.M. 7:00 P.M. | 7.893 8.134 | 1.336 1.308 | 6.557 6.826 | + 3.1 | - 2.1 | + 4.1 | 129 | 139 | 128 |
| { 7:00 P.M. 11:00 P.M. | 8.593 8.996 | 1.387 1.374 | 7.206 7.622 | + 4.7 | - 1.0 | + 5.8 | 134 | 138 | 130 |
| { 11:00 P.M. 4:00 A.M. | 9.718 9.718 | 1.460 1.401 | 8.258 8.317 | 0 | - 4.0 | + 0.7 | 134 | 132 | 129 |

* Percent change in each interval calculated on the amount present at the beginning of that period.

NOTE: Brackets indicate twin-leaves.

The columns in tables 4, 5, and 6 which show the total weights in 30 leaves are self-explanatory. In the central columns in each table the percentage gain or loss in the amount of the constituent is based upon the amount present at the beginning of that period; thus, in table 5, column 9, the dry weight changed from 2.125 g. at 5:30 A.M. to 2.198 g. at 8:00 A.M.; the difference is 0.073 g. which is 3.4 percent of 2.125. In this way the changes during each period may be plotted as has been done, for example, in text figure 3; such a graph is a rate-curve and shows when the rate of change is the highest, and when it reverses in sign, if at all, etc.

TABLE 5. *Diurnal Changes in the Composition of Salvia Leaves. Leaves Below Tip. April 1929 Experiment*

| Time | Total Amount in 30 Leaves | | | | | | Percent Gain (+) or Loss (-) During the Interval * | | | | Relative Amount at End of Interval, Amount at 5:30 A.M. as 100 | | | | |
|------------------|---------------------------|---------------|-------------|--------------|-------------------|------------------|---|------------|-------|--------|---|------------|-------|--------|----------------|
| | Fresh Wt. g. | Dry Wt. g. | Water g. | Starch g. | Sol. Solids g. | Insol. N. mg. | Fresh Wt. | Dry Wt. | Water | Starch | Fresh Wt. | Dry Wt. | Water | Starch | Sol. Solids |
| { 5:30 A.M..... | 17.467 | 2.125 | 15.342 | 0.339 | 0.800 | 102 | | | | | | | | | |
| { 8:00 A.M..... | 17.377 | 2.198 | 15.179 | 0.383 | 0.817 | 99 | -0.5 | + 3.4 | -1.1 | +13.0 | 99 | 103 | 99 | 113 | 102 |
| { 8:00 A.M..... | 15.349 | 1.961 | 13.388 | 0.339 | 0.709 | 95 | | | | | | | | | |
| { 10:30 A.M..... | 15.305 | 2.157 | 13.148 | 0.491 | 0.790 | 93 | -0.3 | +10.0 | -1.8 | +45.0 | 99 | 113 | 97 | 164 | 113 |
| { 10:30 A.M..... | 15.570 | 2.281 | 13.289 | 0.514 | 0.813 | 98 | | | | | | | | | |
| { 1:00 P.M..... | 16.437 | 2.630 | 13.807 | 0.715 | 0.877 | 116 | +5.6 | +15.3 | +3.9 | +39.2 | 105 | 130 | 102 | 228 | 122 |
| { 1:00 P.M..... | 15.893 | 2.491 | 13.402 | 0.756 | 0.883 | 101 | | | | | | | | | |
| { 3:30 P.M..... | 16.552 | 2.630 | 13.922 | 0.766 | 0.907 | 106 | +4.2 | + 5.6 | +3.9 | + 1.4 | 109 | 138 | 106 | 231 | 125 |
| { 3:30 P.M..... | 17.441 | 2.802 | 14.639 | 0.902 | 0.924 | 108 | | | | | | | | | |
| { 7:00 P.M..... | 18.217 | 2.749 | 15.468 | 0.835 | 0.812 | 115 | +4.5 | - 1.9 | +5.7 | - 7.5 | 113 | 135 | 112 | 214 | 110 |
| { 7:00 P.M..... | 17.461 | 2.738 | 14.723 | 0.856 | 0.876 | 112 | | | | | | | | | |
| { 11:00 P.M..... | 17.902 | 2.612 | 15.290 | 0.647 | 0.856 | 107 | +2.5 | - 4.6 | +3.9 | -24.0 | 116 | 129 | 116 | 163 | 107 |
| { 11:00 P.M..... | 19.202 | 2.715 | 16.487 | 0.811 | 0.868 | 112 | | | | | | | | | |
| { 4:00 A.M..... | 19.004 | 2.559 | 16.445 | 0.631 | 0.821 | 111 | -1.0 | - 5.7 | -0.3 | -22.0 | 115 | 122 | 116 | 127 | 102 |

* Percent change in each interval calculated on the amount present at the beginning of that period.

NOTE: Brackets indicate twin-leaves.

TABLE 6. *Diurnal Changes in the Composition of Salvia Leaves. Third Pair (Second Pair of Leaves Below Tip). April 1929 Experiment*

| Time | Total Amount in 30 Leaves | | | | | | | Percent Gain (+) or Loss (-) During the Interval * | | | | Relative Amount at End of Inter- val, Amount at 5:30 A.M. as 100 | | | |
|------------------|---------------------------|---------------|-------------|---------------|-------------------|----------------|------------------|---|------------|-------|--------|---|------------|--------|----------------|
| | Fresh Wt. g. | Dry Wt. g. | Water g. | Starch g.† | Sol. Solids g. | Sol. N. mg. | Insol. N. mg. | Fresh Wt. | Dry Wt. | Water | Starch | Fresh Wt. | Dry Wt. | Starch | Sol. Solids |
| { 5:30 A.M..... | 22.307 | 2.609 | 19.698 | 0.149 | 0.962 | 14.4 | 121 | | | | | | | | |
| { 8:30 A.M..... | 22.101 | 2.694 | 19.407 | 0.156 | 1.001 | 14.7 | 123 | -1.2 | + 3.2 | -1.5 | + 4.7 | 99 | 103 | 105 | 104 |
| { 8:00 A.M..... | 19.792 | 2.395 | 17.397 | 0.152 | 0.817 | 12.5 | 113 | | | | | | | | |
| { 10:30 A.M..... | 19.017 | 2.792 | 16.225 | 0.290 | 0.883 | 13.6 | 117 | -3.9 | +15.9 | -6.7 | +91.0 | 95 | 120 | 201 | 112 |
| { 10:30 A.M..... | 19.564 | 2.778 | 16.786 | 0.290 | 0.963 | 12.8 | 120 | | | | | | | | |
| { 1:00 P.M..... | 20.438 | 3.045 | 17.373 | 0.434 | 0.985 | 15.5 | 137 | +4.5 | + 9.1 | +3.6 | +49.7 | 99 | 131 | 300 | 114 |
| { 1:00 P.M..... | 20.425 | 3.064 | 17.361 | 0.473 | 1.016 | 14.8 | 125 | | | | | | | | |
| { 3:30 P.M..... | 21.272 | 3.220 | 18.052 | 0.542 | 1.046 | 15.7 | 119 | +4.1 | + 5.1 | +4.0 | +14.6 | 103 | 137 | 344 | 117 |
| { 3:30 P.M..... | 22.220 | 3.409 | 18.811 | 0.567 | 1.071 | 18.8 | 133 | | | | | | | | |
| { 7:00 P.M..... | 23.021 | 3.416 | 19.605 | 0.530 | 1.024 | 16.6 | 136 | +3.6 | + 0.2 | +4.2 | - 6.5 | 107 | 138 | 322 | 112 |
| { 7:00 P.M..... | 21.128 | 3.126 | 18.002 | 0.491 | 0.966 | 15.9 | 123 | | | | | | | | |
| { 11:00 P.M..... | 21.537 | 2.999 | 19.538 | 0.394 | 0.971 | 15.9 | 122 | +1.9 | - 4.1 | +8.5 | -19.4 | 109 | 132 | 259 | 113 |
| { 11:00 P.M..... | 21.647 | 3.065 | 18.582 | 0.397 | 1.009 | 15.5 | 123 | | | | | | | | |
| { 4:00 A.M..... | 21.120 | 2.821 | 18.299 | 0.299 | 0.910 | 14.2 | 125 | -2.4 | - 7.9 | -1.5 | -24.9 | 106 | 121 | 195 | 103 |

* Percent change in each interval calculated on the amount present at the beginning of that period.

† By Walton and Coe (14) method.

NOTE: Brackets indicate twin-leaves.

The right hand columns in tables 4, 5, and 6 show the percentage at the end of any given period when the amount at the start of the experiment is placed at 100; thus, in table 4, column 9, the dry weight at 5:30 A.M. is 100, and since the gain from 5:30 to 8:00 was 6.0 percent (see column 6), the amount at 8:00 is 106; and since the gain from 8:00 to 10:30 was 11.4 percent of the amount at 8:00 the percentage at 10:30 with reference to the start was $106 + (0.114 \times 106) = 118$. In this way the values showing the relations between the amount at any time and the amount at the start of the day were obtained (see text figs. 1 and 2).

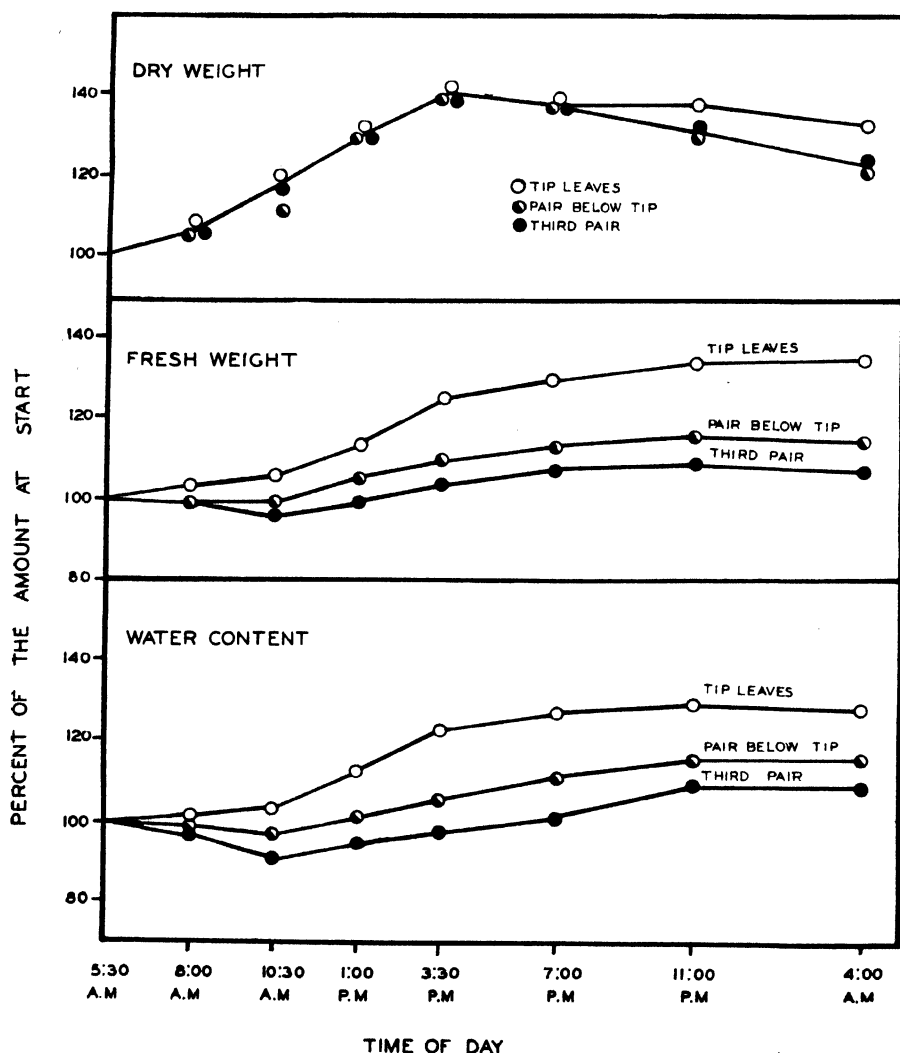
There is a danger in this method of procedure, in that it tends to pyramid values, and to propagate throughout the daily record any erroneous reading that may have been taken. But the occurrence of any large error can be detected by calculating the fresh weight percentage for the constituent at each sampling period. Thus, with the exception of the first and last samples, there are available always two simultaneous samples at each period, and, although these two were obtained by taking leaves from different plants, the fresh weight percentages should be reasonably nearly the same; in this way the occurrence of any large error can be detected, and its propagation throughout the day can be avoided. Although these calculated values do not have the dependability of the original data they show the general change throughout the day, and permit the construction of curves such as text figures 1 and 2, which have been built up into a continuous curve from the step-wise measurements of the individual periods.

Another method of collecting samples so as to avoid this propagation of errors would be to pick at the beginning one leaf from each of all the pairs to be used during the experiment, keeping them in as many groups as there are subsequent sampling periods, and then to pick the corresponding pairs at intervals thereafter. The differences for different periods will then be the total difference over the entire period, and there will be no propagation of error. This has the disadvantage that no samples except the first will be simultaneous, and consequently no opportunity is had to check against accidental errors by computing the fresh weight percentages of the simultaneous samples of non-twin leaves.

Changes in Fresh Weight, Dry Weight, and Water Content

Tables 4, 5, 6 and text figure 1 show an interesting difference in the behavior of the three types of leaves with reference to fresh weight, dry weight, and water content. The tip leaves did not decrease in fresh weight or water content at any time during the day but gained continually; the pair below the tip and the third pair lost water early in the morning up to about 10:30, at which time they began to gain in water, but the pair below the tip did not recover the water previously lost until about 1:00 P.M., and the third pair not until about 5:00 P.M. The fresh weight change reflected the moisture change, being lowered somewhat in the early forenoon

and rising again during the night. Although the three types of leaves differed with respect to fresh weight and moisture changes, the dry weight changes were very similar (see table 4, column 6; table 5, column 9; table 6, column 10, and text fig. 1). Even though the leaf weights of the types were quite different, the percentage increases during the first few periods

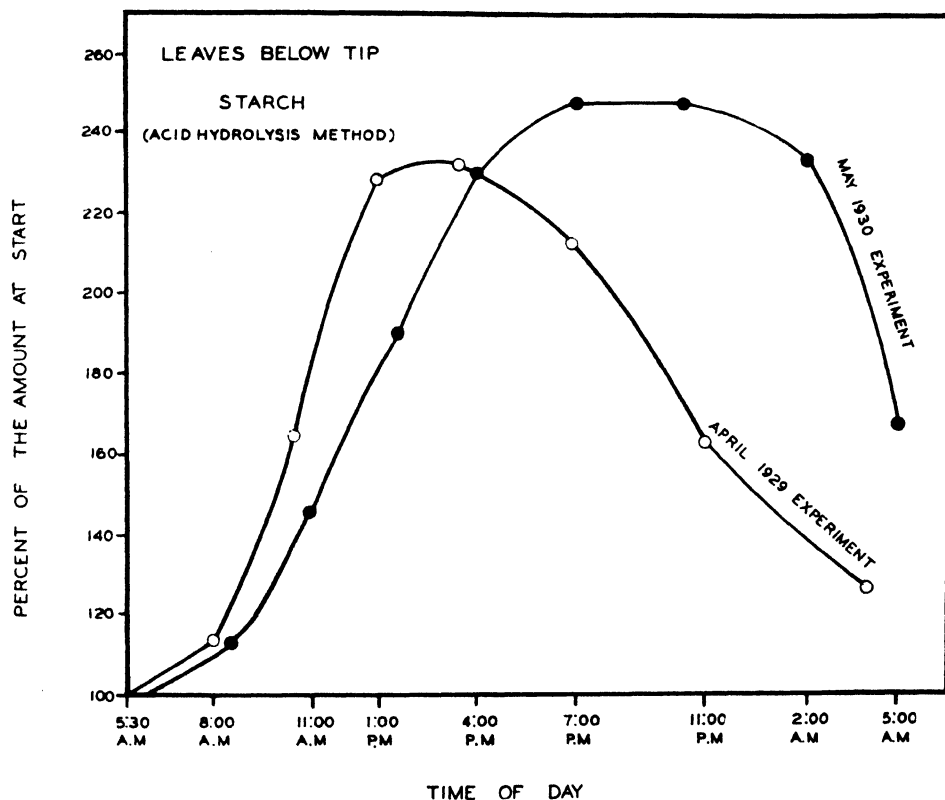


TEXT FIG. 1. Amount present at the end of each interval with reference to the amount present at the start. Notice differences in the behavior of tip leaves, leaves below the tip, and the third pair of leaves, *i.e.*, second pair below the tip. *Salvia splendens* Ker.

were similar numerically; during the night, however, the fall in dry weight was not so fast in the tip leaves as in the other types (see text fig. 1). All three types had higher dry weights in the early morning of the second day than they had at the start of the experiment, whether the calculation is made on the twin leaf basis or on the percentage of the fresh weight basis.

Starch

Although the term starch is applied throughout this paper, the method mainly used was that of acid-hydrolysis (see I, p. 95), and consequently the determination includes not only true starch but also all other compounds that hydrolyze with dilute acid to give copper reducing substances. In the present experiments the purpose was to test the use of the twin-leaf method for studying the changes of substances, and emphasis was not put on the nature of the substances themselves; consequently this grouping of substances will not seriously disturb the general conclusions. The Walton



TEXT FIG. 2. Starch by acid-hydrolysis method in leaves below the tip with reference to the amount present at the start. Notice that time of attainment of the maximum and of the beginning of rapid translocation was early in the 1929 experiment and late in the 1930 experiment. *Salvia splendens* Ker.

and Coe (14) method, which eliminates interfering polysaccharids, was used in one test (third pair of leaves in the 1929 experiment), but there was doubt as to whether the enzyme was bringing about complete hydrolysis of the starch. Subsequent determinations showed about 25 percent lower values by the Walton and Coe procedure than by the acid-hydrolysis method. No doubt a study of changes in the non-starch acid-hydrolyzable polysaccharids would give interesting results, and it appears likely that the twin-leaf method would be well suited for this purpose.

The quantity of starch (or the acid-hydrolyzable substances) found in each sample is shown in table 5, column 5, and table 6, column 5. The percentage increases during each experimental period are shown in column 11, table 5, and in column 12, table 6. The greatest percentage increases occurred in the period from about 8:00 to 10:30 A.M., but the maximum amount of starch in the tissue was not reached until later in the day, about 1:00 to 3:30 P.M.

In text figure 2 will be found the relative amounts of starch present in the leaves at the various intervals during the day with reference to the amount present in the early morning.

The data in tables 5, 6, and 7*B* show increases during a 2.5 hour period of 30 to 90 percent of the amount of acid-hydrolyzable substances present at the beginning of the period; this indicates that it would be possible to measure this increase over a much shorter period, possibly during a 30 minute interval.

Soluble Solids

The weights of material soluble under the conditions of extraction used in these experiments are shown in table 5, column 6, and table 6, column 6; they are of interest in showing that the soluble solids made a complete excursion during the 24 hour period, increasing in amount up to about 2:00 P.M., and then decreasing so that the amount present the next morning was about the same as that at the beginning of the experiment.

Insoluble Nitrogen

The changes in the amounts of nitrogen in the insoluble fraction were so small that it can not be definitely stated whether any change at all occurred. As shown in table 5, column 7, and in table 6, column 8, the absolute amounts in 30 leaves underwent small changes during each experimental period but it seems unlikely that these differences are significant. Furthermore, when these amounts are calculated on the percentage of fresh weight basis, it is found that the range of values including both types of leaves and at all sampling periods throughout the day was only from 0.54 percent to 0.71 percent of the fresh weight. There was a tendency for slightly higher values at about noon and lower values in the early morning. But it would require larger samples with the resulting smaller experimental errors to obtain dependable values for showing the change in insoluble nitrogen.

Results of the May 1930 Experiment

The results of the May 1930 experiment are shown in tables 7*A* and 7*B*. Table 7*A* gives the total amounts of substances found in the entire sample at each experimental period, and table 7*B* shows the percentage change during each period, and the relation of the value at any time to that at the beginning of the experiment.

TABLE 7 A. *Changes in Salvia Leaves During a Day and Night. May 1930 Experiment*

| Time | Tip Leaves | Leaves Below Tip | | | Third Pair (Second Pair Below Tip) | | | | | |
|-------------------|--------------------|--------------------|-----------|---------------|------------------------------------|------------|----------|-----------|-------------|---------------|
| | Total in 20 Leaves | Total in 20 Leaves | | | Total in 15 Leaves | | | | | |
| | Fresh Wt. g. | Fresh Wt. g. | Starch g. | Insol. N. mg. | Fresh Wt. g. | Dry Wt. g. | Water g. | Starch g. | Sol. N. mg. | Insol. N. mg. |
| { 6:00 A.M. | 4.235 | 9.575 | 0.148 | 52 | 8.494 | 1.048 | 7.446 | 0.170 | 4.6 | 39 |
| { 8:30 A.M. | 3.985 | 8.925 | 0.166 | 54 | 7.631 | 1.083 | 6.548 | 0.188 | 4.2 | 39 |
| { 8:30 A.M. | 4.085 | 9.860 | 0.160 | 60 | 9.253 | 1.261 | 7.992 | 0.196 | 4.3 | 47 |
| { 11:00 A.M. | 4.135 | 9.948 | 0.207 | 61 | 9.177 | 1.353 | 7.824 | 0.283 | 5.0 | 49 |
| { 11:00 A.M. | 3.716 | 8.945 | 0.178 | 57 | 7.978 | 1.164 | 6.814 | 0.200 | 5.8 | 43 |
| { 1:30 P.M. | 3.859 | 9.324 | 0.232 | 60 | 8.064 | 1.282 | 6.782 | 0.260 | 5.2 | 45 |
| { 1:30 P.M. | 3.632 | 9.315 | 0.218 | 58 | 8.471 | 1.366 | 7.105 | 0.289 | 5.0 | 46 |
| { 4:00 P.M. | 3.865 | 9.723 | 0.264 | lost | 8.742 | 1.491 | 7.251 | 0.307 | lost | 48 |
| { 4:00 P.M. | 3.213 | 8.562 | 0.238 | 56 | 8.347 | 1.409 | 6.938 | 0.296 | 4.6 | 44 |
| { 7:00 P.M. | 3.340 | 8.862 | 0.257 | 56 | 8.619 | 1.479 | 7.140 | 0.296 | 4.9 | 45 |
| { 7:00 P.M. | 3.515 | 7.902 | 0.227 | 49 | 7.814 | 1.265 | 6.549 | 0.273 | 4.2 | 41 |
| { 10:30 P.M. | 3.659 | 8.110 | 0.227 | 49 | 7.676 | 1.234 | 6.442 | 0.273 | 4.0 | 40 |
| { 10:30 P.M. | 3.669 | 8.562 | 0.196 | lost | 7.896 | 1.255 | 6.641 | 0.282 | 4.5 | 41 |
| { 2:00 A.M. | 3.654 | 8.453 | 0.180 | 53 | 7.649 | 1.131 | 6.518 | 0.208 | 4.1 | 41 |
| { 2:00 A.M. | 3.784 | 9.137 | 0.212 | 56 | 8.637 | 1.333 | 7.304 | 0.242 | 3.4 | 46 |
| { 5:00 A.M. | 3.952 | 9.247 | 0.151 | 52 | 8.697 | 1.241 | 7.456 | 0.197 | 4.4 | 44 |

NOTE: Brackets indicate twin-leaves.

Fresh Weight

Columns 2, 3, and 6 in table 7A, and columns 2, 3, and 5 in table 7B show that the fresh weight behavior was similar in most respects to that noted in the 1929 experiment. The fresh weight losses in the early morning hours were greater in the 1930 experiment than in that of 1929, and the gain during the night was not as great. The percentage changes during the different periods are shown for the third pair of leaves in text figure 3.

Dry Weight

The dry weight values for the third pair of leaves are shown in table 7A, column 7, and in table 7B, columns 6 and 13. They are of special interest in comparison with the results from the 1929 experiment in showing maximum dry weight values much later in the day. Thus, in 1929 this maximum was reached by mid-afternoon but in 1930 it did not occur until about 7:00 P.M. The percentage changes in dry weight during different intervals are shown for the third pair of leaves in text figure 3.

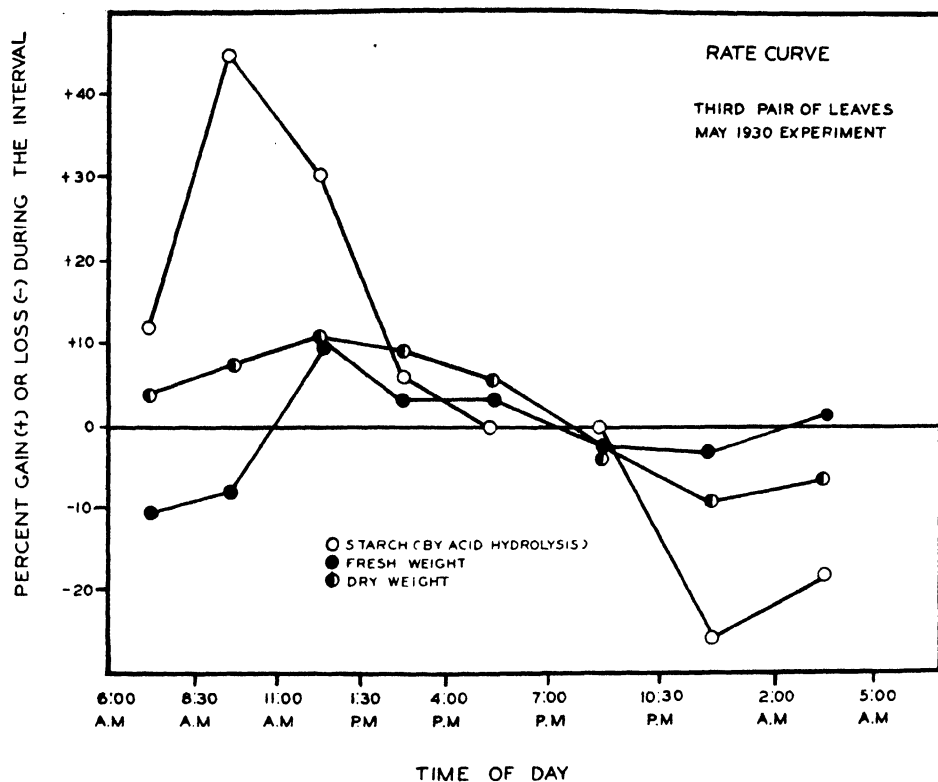
TABLE 7 B. *Percentage Changes and Relative Amounts Present at Intervals, as Calculated from Data in Table 7 A*

| Time Interval | Percent Gain (+) or Loss (-) During Each Interval * | | | | | | | Relative Amount Present at End of Period, Amount at 6:00 A.M. as 100 | | | | | |
|-----------------------------|---|------------------|--------|--------------|------------|-------|--------|---|---------------------|--------|--------------|------------|--------|
| | Tip Leaves | Leaves Below Tip | | Third Pair | | | | Tip Leaves | Leaves Below Tip | | Third Pair | | |
| | Fresh Wt. | Fresh Wt. | Starch | Fresh Wt. | Dry Wt. | Water | Starch | Fresh Wt. | Fresh Wt. | Starch | Fresh Wt. | Dry Wt. | Starch |
| 6:00 to 8:30 A.M..... | -6.0 | -6.8 | +12.2 | -10.2 | + 3.3 | -12.1 | +11.8 | 94 | 93 | 112 | 90 | 103 | 112 |
| 8:30 to 11:00 A.M..... | +1.2 | +0.9 | +29.4 | - 8.2 | + 7.3 | - 2.1 | +44.4 | 95 | 94 | 145 | 83 | 111 | 161 |
| 11:00 A.M. to 1:30 P.M..... | +3.8 | +4.2 | +30.4 | +10.8 | +10.2 | - 0.5 | +30.0 | 99 | 98 | 189 | 92 | 122 | 210 |
| 1:30 to 4:00 P.M..... | +6.4 | +4.4 | +21.0 | + 3.2 | + 9.1 | + 2.1 | + 6.2 | 105 | 102 | 229 | 94 | 133 | 223 |
| 4:00 to 7:00 P.M..... | +3.9 | +3.5 | + 8.0 | + 3.3 | + 5.0 | + 2.9 | 0 | 109 | 106 | 247 | 98 | 140 | 223 |
| 7:00 to 10:30 P.M..... | +4.1 | +2.6 | + 2.7 | - 1.8 | - 2.4 | - 1.6 | 0 | 113 | 109 | 247 | 96 | 131 | 223 |
| 10:30 to 2:00 A.M..... | -0.4 | -1.3 | - 8.2 | - 3.1 | - 9.9 | - 1.9 | -26.0 | 113 | 107 | 233 | 93 | 118 | 217 |
| 2:00 to 5:00 A.M..... | +4.4 | +1.2 | -28.8 | + 0.7 | - 6.9 | + 2.1 | -18.6 | 118 | 109 | 166 | 93 | 109 | 177 |

* Percent change during each interval calculated on the amount present at the beginning of that period.

Water Content

This was obtained in the 1930 experiment only for the third pair of leaves (second pair below tip, see table 7A, column 8, and table 7B, column 7). Losses of water were high until 11:00 A.M., at which time the water



TEXT FIG. 3. Rate curve; gain or loss during each period expressed as the percent of the amount present at the beginning of the period, and plotted at the middle of each period. All three curves for the third pair of leaves, i.e., second pair below tip. *Salvia splendens* Ker.

content began to gain; but these leaves did not recover their original water content during the night as was the case in the 1929 experiment.

Starch (Acid-hydrolyzable Polysaccharids)

The data for the pair of leaves below the tip and for the third pair of leaves are shown in table 7A, columns 4 and 9, and in table 7B, columns 4, 8, 11, and 14. The percentage gains during each 2.5 hour period were again large in the forenoon, the gains being 30 to 44 per cent of the amount present at the beginning of each period. The results of the 1930 experiment differed from those of 1929 in the time during the night at which the losses began to take place. In the 1929 series this reduction in the amount of starch started in mid-afternoon (see text fig. 2), but in the 1930 series losses in starch did not begin until about midnight (see text fig. 2); the

starch values remained nearly constant from about 4:00 P.M. until about 10:30 P.M.

Insoluble Nitrogen

In the 1930 as in the 1929 experiments the data show very little change in the amount of nitrogen in the insoluble portion. The absolute amounts in the samples at the beginning and at the end of each period fail to show any clear gains or losses that can not be accounted for as experimental errors, and the percentage of the fresh weight showed nearly the same values throughout the day. Here again the values tend to be slightly higher during the middle of the day but the difference is not great enough to be conclusive.

Sugar

The preliminary experiments in 1929 indicated that *Salvia* leaves were very low in sugar, this constituent being about 0.1 to 0.3 percent of the fresh weight. In the 1930 experiments an attempt was made to measure the sugar change during the day by means of the Somogyi (11) modification of the Shaffer and Hartman (10) method. This is applicable to amounts of sugar varying from zero to two milligrams in a five cc. sample. The sugar values obtained in the 1930 experiments for the various samples throughout the day are shown in table 8 which gives not only the absolute

TABLE 8. *Diurnal Variation of Sugar in Leaves of Salvia May 1930 Experiment*

| Time of Day | Tip Leaves | | | | Third Pair | | | |
|-------------|------------------------------|-------------------------|------------------------------|-------------------------|------------------------------|-------------------------|------------------------------|-------------------------|
| | Reducing Sugar | | Sucrose * | | Reducing Sugar | | Sucrose * | |
| | Total in 20 Leaves mg. | % of Fresh Weight | Total in 20 Leaves mg. | % of Fresh Weight | Total in 15 Leaves mg. | % of Fresh Weight | Total in 15 Leaves mg. | % of Fresh Weight |
| 6:00 A.M. | 7.2 | 0.17 | 4.3 | 0.10 | 8.6 | 0.11 | 7.8 | 0.09 |
| 8:30 A.M. | 8.5 | 0.21 | 6.3 | 0.16 | 9.6 | 0.13 | 10.0 | 0.13 |
| 8:30 A.M. | 9.9 | 0.24 | 7.5 | 0.15 | 12.5 | 0.14 | 11.1 | 0.12 |
| 11:00 A.M. | 10.3 | 0.25 | 9.0 | 0.22 | 16.1 | 0.18 | 13.8 | 0.15 |
| 11:00 A.M. | 9.9 | 0.27 | 8.1 | 0.22 | 13.4 | 0.17 | 13.2 | 0.17 |
| 1:30 P.M. | 8.6 | 0.23 | 7.8 | 0.20 | 12.5 | 0.14 | 9.6 | 0.12 |
| 1:30 P.M. | 8.8 | 0.24 | 7.1 | 0.20 | 13.7 | 0.16 | 13.2 | 0.16 |
| 4:00 P.M. | 8.5 | 0.24 | 9.2 | 0.24 | 12.1 | 0.14 | 14.2 | 0.16 |
| 4:00 P.M. | 7.0 | 0.22 | 9.4 | 0.29 | 12.6 | 0.15 | 13.6 | 0.16 |
| 7:00 P.M. | 6.9 | 0.21 | 10.6 | 0.32 | 12.6 | 0.15 | 15.5 | 0.18 |
| 7:00 P.M. | 7.4 | 0.21 | 9.9 | 0.28 | 8.2 | 0.12 | 14.0 | 0.18 |
| 10:30 P.M. | 7.3 | 0.20 | 7.4 | 0.20 | 10.1 | 0.14 | 13.0 | 0.17 |
| 10:30 P.M. | 6.5 | 0.18 | 9.7 | 0.26 | 8.3 | 0.11 | 14.4 | 0.18 |
| 2:00 A.M. | 6.0 | 0.16 | 6.5 | 0.18 | 7.4 | 0.10 | 9.1 | 0.12 |
| 2:00 A.M. | 6.7 | 0.18 | 6.3 | 0.17 | 8.8 | 0.11 | 13.8 | 0.16 |
| 5:00 A.M. | 7.1 | 0.18 | 4.3 | 0.11 | 8.9 | 0.11 | 7.8 | 0.09 |

* By acid inversion in the cold, see 1, p. 95.

amounts in the entire sample but also the fresh weight percentages. It is seen that only small changes occurred. The reducing sugar values gave increases up to about 11:00 A.M. and then fell off toward the next morning. The sucrose values are subject to greater error since they are arrived at by means of the difference of two measurements; but, as a whole, low values were obtained in the early morning samples, with higher values about 4:00 P.M.

It may be questioned whether these observed differences are real, in view of the small amounts present and the large percentage error involved in their determination. The percentage of the fresh weight varied only between the limits of 0.11 percent and 0.27 percent for the reducing sugar, and between 0.09 percent and 0.32 percent for the sucrose. Compared, therefore, with the changes that were observed in fresh weight, dry weight, starch, etc., the sugars have shown very little fluctuations during the day.

It should be stated that these sugar determinations were made with uncleared solutions and represent, therefore, not merely sugar but all other substances that reduce copper under these conditions. It was not found feasible to clear with lead acetate, since the volume of liquid available for the test was small and the losses in amounts of liquid resulting from the procedures in leading and deleading were large. Preliminary tests indicated that leading and deleading decreased the apparent sugar content by about one-fifth or one-fourth.

Dry Weight Increases per Square Meter of Leaf Surface per Hour

Time was not available for taking the leaf areas of all the samples in these series of measurements. But, to permit a comparison of these measurements with previous work on diurnal changes in which the results were always expressed on the leaf area basis, the general relation between the leaf weight and area was established. Thus, in table 9 are shown the

TABLE 9. *Relation Between Fresh Weight and Area of Salvia Leaves*

| Fresh Weight of Leaf, grams | Area in sq. cm. | Fresh Weight of Leaf, grams | Area in sq. cm. |
|--------------------------------|--------------------|--------------------------------|--------------------|
| 0.86 | 19.8 | 0.30 | 8.2 |
| 0.60 | 13.7 | 0.08 | 2.7 |
| 0.38 | 9.9 | 0.13 | 3.9 |
| 0.27 | 8.2 | 0.30 | 7.8 |
| 0.16 | 5.7 | 0.27 | 8.4 |
| 0.17 | 5.3 | 0.48 | 12.6 |
| 0.52 | 13.0 | 0.37 | 10.7 |
| 0.43 | 11.4 | 0.16 | 5.3 |
| 0.52 | 13.7 | 0.22 | 6.9 |
| 0.68 | 19.0 | 0.46 | 12.0 |

NOTE: The weights and areas of leaves were recorded in the above table in the order in which the measurements were made. From this table a graph (not shown in this paper) was prepared, giving the relation between weight and area. From this graph the average areas of the leaves of the various samples were calculated for use in table 10. Small leaves have a greater leaf area per fresh weight than large leaves, e.g., leaves that were 0.1 gram in weight gave areas of about 3.1 sq. cm. while those with weights of 0.3 g. and 0.5 g. gave areas of about 8.3 and 13.0 sq. cm., respectively.

fresh weights and leaf areas of 20 *Salvia* leaves of various sizes. From these measurements a graph (not shown) was prepared from which the average leaf weights in the various samples were translated into average leaf areas, at least with fair accuracy. The corresponding gain in dry weight per leaf area during each interval was calculated for the leaves below the tip in the 1929 experiment, and for the third pair of leaves in both years. The results are shown in table 10 which shows the gain in grams per square

TABLE 10. *Dry Weight Increase per Square Meter per Hour*

| April 1929 Experiment | | | May 1930 Experiment | |
|-----------------------|---|---|---------------------|---|
| Time Interval | Leaves Below Tip g. per sq. m. per hr. | Third Pair g. per sq. m. per hr. | Time Interval | Third Pair g. per sq. m. per hr. |
| 5:30 to 8:00 A.M. | 0.50 | 0.33 | 6:00 to 8:30 A.M. | 0.66 |
| 8:00 to 10:30 A.M. | 1.16 | 2.78 | 8:30 to 11:00 A.M. | 1.58 |
| 10:30 to 1:00 P.M. | 2.68 | 1.94 | 11:00 to 1:30 P.M. | 2.17 |
| 1:00 to 3:30 P.M. | 1.09 | 1.00 | 1:30 to 4:00 P.M. | 2.28 |
| 3:30 to 7:00 P.M. | 0.39 | 0.30 | 4:00 to 7:00 P.M. | 1.05 |
| Average | 1.16 | 1.27 | Average | 1.54 |

meter of leaf area per hour during each interval in which gains in dry weight were made. It is seen that a series of values was obtained showing how the rate changed from one period to another. Thus, in the 1929 experiment, with the leaves below the tip, the successive gains were 0.5 gram per square meter per hour during the period from 5:30 to 8:00 A.M., 1.16 from 8:00 to 10:00 A.M., 2.68 from 10:30 A.M. to 1:00 P.M., 1.09 from 1:00 to 3:30 P.M., and 0.39 from 3:30 to 7:00 P.M. Thereafter the dry weight decreased. It is interesting to compare the results in the two different years, the maximum rate of gain occurring later in the day in the 1930 than in the 1929 experiments; *e.g.*, for the third pair of leaves the highest rate was between 8:00 and 10:30 A.M. in 1929, and between 1:30 and 4:00 in the 1930 experiments.

We may compare these values with previous measurements of dry weight increase. Kostytschew (8, p. 177) gives the amounts per square meter per hour for different species, and in his list the values range from 1.00 to 2.37. The bottom line in table 10 shows that the average values in the present experiments (1.16 to 1.54 g. per sq. m. per hr.) come within the range of the Kostytschew values.

The gains in dry weight per leaf area are usually given as average values over a considerable period of time, often for a ten hour period, but the details in table 10 are of much greater interest, since they show not only the average over a considerable period but also show the values for each interval. It is seen that the rate over a short interval may be more than twice the average rate over a long period; and probably if suitable

conditions as to starch depletion before the start of the experiment were provided, even larger differences between gains during short exposures and average gains over a long period would be found.

DISCUSSION

It is likely that Sachs tested the opposite leaf possibility in connection with the early experiments on this subject, since he speaks (9, p. 7) of the use in some cases of opposite leaflets of compound leaves, but no detailed data on the point have yet come to the writer's notice; also Broocks (2) when dealing with plants "mit gefiederten Blättern (Bohne, Kartoffel)," used opposite leaflets, but there is no evidence that he regarded this as an improvement. Perhaps this method was tested and discarded by them for the reason that the symmetry error was found to be higher with opposite leaves than with opposite halves. They could not foresee, of course, that in later years there would be brought forward objections which would suggest the need of sacrificing accuracy in the sample weight in order to attain an advantage in another direction.

Even though it may be shown in the future that there is greater uniformity in opposite halves than in opposite leaves, we should not merely on that account condemn the twin-leaf method. It has an important advantage in that the errors arising from mutilation of the leaf are much reduced. We can not say at present that the cutting of the petiole has no effect at all upon the opposite leaf, but we can reasonably expect that the effect is small because of the small amount of tissue involved in cutting through the petiole, and because of the distance to the opposite leaf blade whose metabolism must be affected to bring about an error in the method.

The suggestion that the opposite halves of a leaf are more nearly in the same physiological condition than any two leaves upon the plant, does not appear to represent the situation correctly. This may be true when the leaf is intact, but when one half is cut away the physiological condition of the other half is so seriously disturbed that the previous advantage in this respect is no longer present.

There is a restriction in the number of kinds of plants with which the twin-leaf method can be used. Only those with opposite leaves or leaflets are available, and, of these, only those showing a sufficient uniformity in size and chemical composition for the purposes of the experiment. But it is believed that there are many such, and since in certain types of experiments the species to be used can be deliberately chosen, it is possible that the method can find a good field of usefulness.

SUMMARY

1. Because of the objections that have been made against Sachs' half-leaf method of measuring changes in leaves during a definite time interval, attention was turned to the possibility of using the pair of leaves of species

having opposite leaves or leaflets, the plan being to take one leaf of the pair at the beginning of the period and the other one at the end. It is suggested that this be called the "twin-leaf" method to distinguish it from the half-leaf method. An alternative name is the "opposite-leaf" method.

2. In order to determine the extent of the variation in opposite leaves, samples of single pairs and of composite samples including several leaves were taken, fresh and dry weights were obtained, and in some cases chemical analyses for various constituents were made.

3. Tests of different species showed favorable results with several, the error involved in the assumption that the weights of the opposite leaves were equal amounting in *Salvia splendens* to about five percent, which would represent an error of about one percent on a composite sample of 25 leaves and of about two percent on a sample of nine.

4. The method was then applied to the determination of diurnal changes in the leaf blades of *Salvia*. Samples were taken on April 3, 1929, at 5:30, 8:00, 10:30 A.M., 1:00, 3:30, 7:00, 11:00 P.M., and 4:00 A.M.; 30 leaves were taken at the beginning of each period and the opposite leaves in each pair at the end. Another series was carried through the 24 hour period on May 12, 1930, samples being taken at 6:00, 8:30, 11:00 A.M., 1:30, 4:00, 7:00, 10:30 P.M., 2:00 and 5:00 A.M., 15 to 20 leaves being taken at each period and all samples being made comparable by the use of opposite (twin) leaves. In both series leaves of three types were collected: tip leaves, leaves below the tip, and the third pair (*i.e.*, second pair of leaves below the tip). The three types of leaves were collected and analyzed separately.

5. The analytical data for the various samples at each period included fresh weight, dry weight, water content, starch, soluble solids, insoluble nitrogen, etc. The tables and graphs show the absolute amounts of material present in each sample, the percentage gain or loss during each period, and the relative amount of each constituent at any time with respect to the amount present in the first sample taken in the early morning.

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OXYGEN REQUIREMENTS FOR ROOT GROWTH OF CUTTINGS IN WATER¹

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INTRODUCTION

Those who attempt to review the literature on aeration as affecting plant growth find it varied and voluminous. Many reports are conflicting as might be expected in so large a field. Some phases of the subject, such as oxygen requirement for growth in liquid media, have not been extensively investigated. The present paper shows some of the effects of known amounts of dissolved oxygen on growth of roots from cuttings.

Livingston and Free (10), using sealed soil containers which could be auto-irrigated and aerated, concluded that plants vary in their requirements for oxygen, willow being a low and coleus a high oxygen type. Complete deprivation of oxygen caused sensitive species like coleus and heliotrope to wilt. The roots failed to take up water and the plants soon died. Cannon (3) in 1915 noted a relationship between moisture, aeration, and temperature as environmental factors which control the distribution of plants.

Free (8) found that buckwheat in culture solutions was not improved by aeration with air, oxygen, or nitrogen. It was not injured by nitrogen but was killed when aerated with carbon dioxide. Cannon (4) in 1925 showed that the requirements of cotton for oxygen varied with the temperature. Growth was normal at 21° C. when the air surrounding the roots had only 2.6 percent oxygen, while the plants in this same amount of oxygen but at 28° C. gave approximately one-fourth of normal growth. Corn at 18° C. or higher required more than 10 percent oxygen in the air surrounding the roots. When the growth rate of plants was normal for a given concentration of oxygen, the addition of more of this gas did not further increase growth. Emerson (7) found the subterranean systems of plants growing on floating bog mats to be very superficial and nearly all above the water. He thought that some of this superficial development might have been due to toxic materials in bog water, but that oxygen doubtless played a part. Bergman (1) observed that roots of land plants do not live under prolonged submergence. The roots soon die and new ones are developed from the

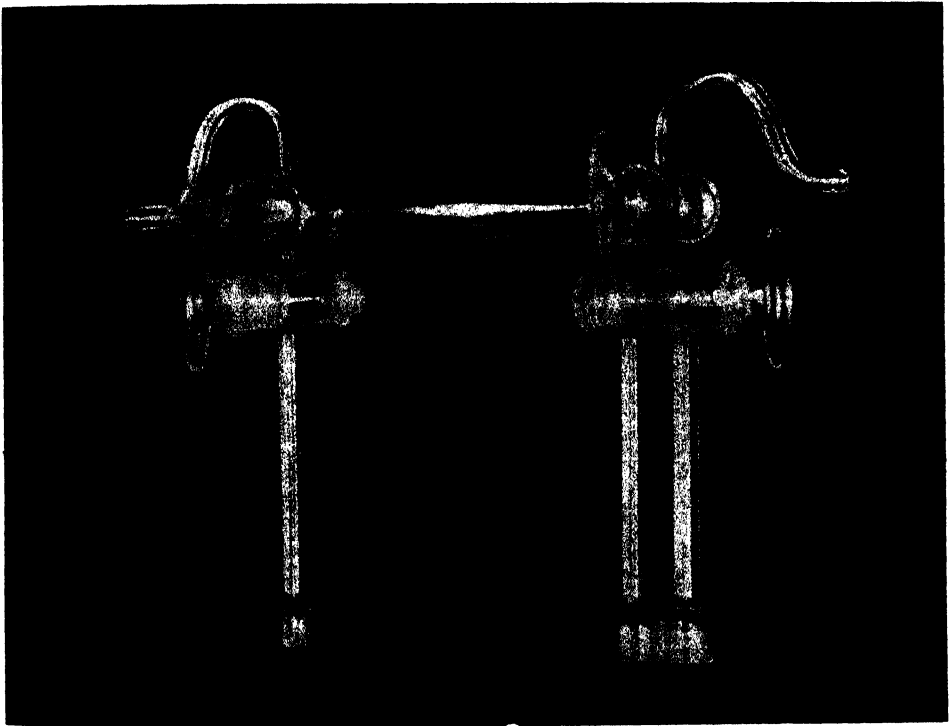
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stem near the surface of the water. When, however, the water was aerated the roots were able to endure submergence, though some retardation was noted. In nature the oxygen content of swamp water decreased from the *Carex* stage to the *Chamaedaphne-Andromeda* stage. Ecesis, he concludes, can occur only when the oxygen requirements are satisfied. Bergman (2) noted that the oxygen content of water where cranberry plants were submerged varied on cloudy and clear days. Shaded tubs containing submerged plants had less oxygen than controls in direct sunlight.

In 1921 Clements (5) published a monograph in which he summarized approximately 700 papers dealing with aeration and air content. With this publication at hand there is little need in this paper for further references to literature. The data reported herewith are primarily to show the effect of various concentrations of oxygen on the production of roots by cuttings.

METHODS

Cuttings were placed in water of different depths. The containers used were glass cylinders 9 inches in height by $1\frac{1}{2}$ inches in diameter or large



TEXT FIG. 1. Apparatus used for micro-determination of oxygen in water.

test tubes 16 inches by 2 inches. A complete experiment usually consisted of aerated and not aerated cuttings in shallow, medium deep, and deep water. Aeration was accomplished by bubbling air or oxygen from cylinders

through the water. Five cc. samples of water were withdrawn from various depths and analyzed for oxygen.

The analyses² were made with a specially designed apparatus described by Thompson and Miller (12). A picture of this apparatus may be seen in text figure 1.

In a few cases oxidizing compounds such as potassium permanganate and hydrogen peroxid were added in different amounts to tap water in which the cuttings were grown. Additional amounts of these chemicals were added at regular intervals.

Tap water was used in most cases. When it was necessary to start with water that was low in oxygen the tap water was first boiled. In some cases, paraffin oil was placed over the surface of the water to decrease absorption of oxygen from the air.

Where light appeared to be a factor in controlling root development or oxygen content the tubes were either wrapped with black paper or placed in a dark room.

Any variations in the methods are described in connection with the report of results.

MATERIAL

Salix pendula (willow), *Forsythia intermedia*, rose (Dorothy Perkins), *Salvia splendens*, *Coleus Blumei*, *Hedera helix* (English ivy), *Lycopersicum esculentum* (Bonny Best tomato), *Ligustrum ovalifolium* (privet), *Philadelphus* sp., *Chrysanthemum* sp., *Prunus tomentosa*, *Portulaca oleracea* constituted the main types that were tested. Long cuttings were used so that they could be placed in deep or shallow water. Both leafy shoots and dormant leafless stems were tested in the course of a year.

RESULTS

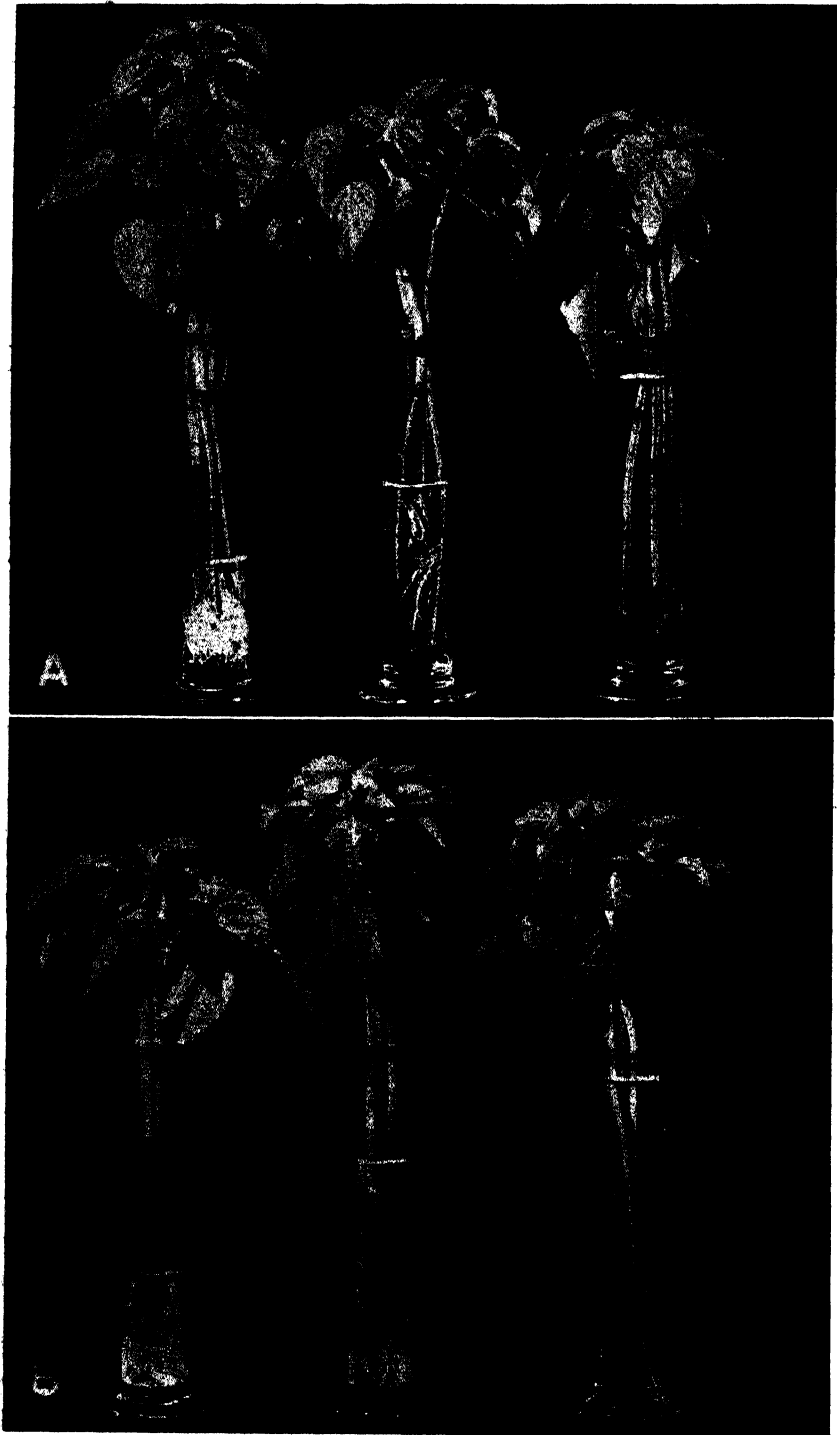
Four long *Salvia splendens* cuttings were placed in each of 12 cylinders so that the basal ends rested on the bottom and the leaves extended above the glass. The cylinders were then divided into four lots of three each for various treatments as shown by the following plan:

² The method of procedure up to the point of titration was well described by Thompson and Miller (12) and for that reason is omitted from this paper. The iodine liberated was titrated with N/500 sodium thiosulphate. According to Scott (11) 1 cc. of N/40 sodium thiosulphate is equivalent to 0.2 milligram oxygen by weight or 0.1395 cc. oxygen by volume under standard conditions. Then 1 cc. of N/500 sodium thiosulphate would be the equivalent of .000016 g. of oxygen. On a 5 cc. sample, which represents the volume analyzed with our apparatus, 1 cc. of N/500 sodium thiosulphate = .000016 \times 100/5 or .00032 percent of oxygen or 3.2 p.p.m. To calculate the parts per million of oxygen in the 5 cc. sample multiply the number of cc. of sodium thiosulphate used in titration by the factor 3.2.

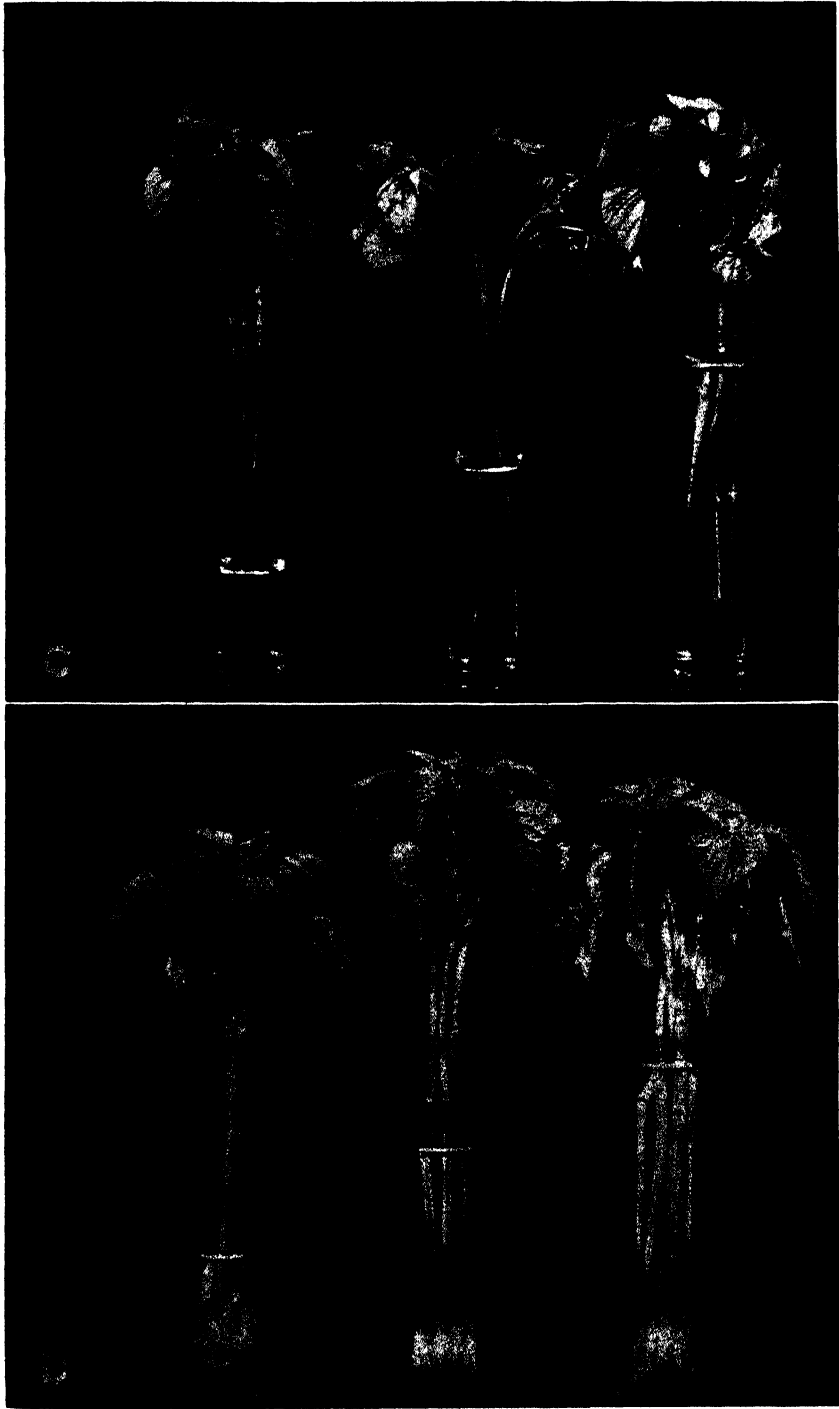
| Lot | Depth of Water Indicated in Inches | Treatment |
|------------------------|---------------------------------------|---|
| Lot A, cylinder 1..... | 1½ | Tap water not aerated |
| Lot A, cylinder 2..... | 4½ | |
| Lot A, cylinder 3..... | 7 | |
| Lot B, cylinder 1..... | 1½ | Tap water aerated with oxygen |
| Lot B, cylinder 2..... | 4½ | |
| Lot B, cylinder 3..... | 7 | |
| Lot C, cylinder 1..... | 1½ | Water was boiled then oiled with paraffin oil to prevent oxygen absorption from the air |
| Lot C, cylinder 2..... | 4½ | |
| Lot C, cylinder 3..... | 7 | |
| Lot D, cylinder 1..... | 1½ | Water was boiled then oiled with paraffin oil and aerated with oxygen |
| Lot D, cylinder 2..... | 4½ | |
| Lot D, cylinder 3..... | 7 | |

The water used in two lots was boiled so as to lower its oxygen content at the beginning of the experiment. In two lots the water was aerated with oxygen from a tank through glass tubes extending to the bottom of the water column. Also in two lots paraffin oil was placed on the surface of the water to decrease the absorption of oxygen from the air. The water levels were kept constant by means of siphons from reservoirs. The results of the experiment are shown in text figures 2 and 3. Rooting occurred in lot A at the base of the cutting in shallow tap water, but in the deeper water roots appeared some distance above the base near the surface of the water. Cuttings in the deepest water did not root. Lot B which was the same except that the water was aerated showed rooting at the base of all the cuttings even in the deepest water. Lot C in water which had been boiled and oiled produced no roots, but Lot D which had water that had been boiled, oiled, and then aerated produced roots at the base in all depths of water. Where aeration was maintained salvia cuttings rooted regularly at the base, whereas rooting occurred above the base in four inches of non-aerated water. At the time this experiment was in progress equipment for determining small quantities of oxygen in water was not available, but there was strong evidence that oxygen was the limiting factor. Accordingly, in later experiments a method was worked out whereby 5 cc. samples of water could be drawn out of the cylinders and analyzed quantitatively for oxygen. The method used was a modification of the Winkler (13) method with an apparatus described by Thompson and Miller (12). With this method it was possible to withdraw and analyze a sample from any point along the stem with very little mixing of the water.

In order to get quantitative measurements of minimum oxygen requirements for root growth, some cuttings were selected from plant types like willow which have the capacity to root at various places along the stem. These cuttings were grown in 16" glass tubes as shown in text figure 4. The response was somewhat similar to that of salvia in that there was a

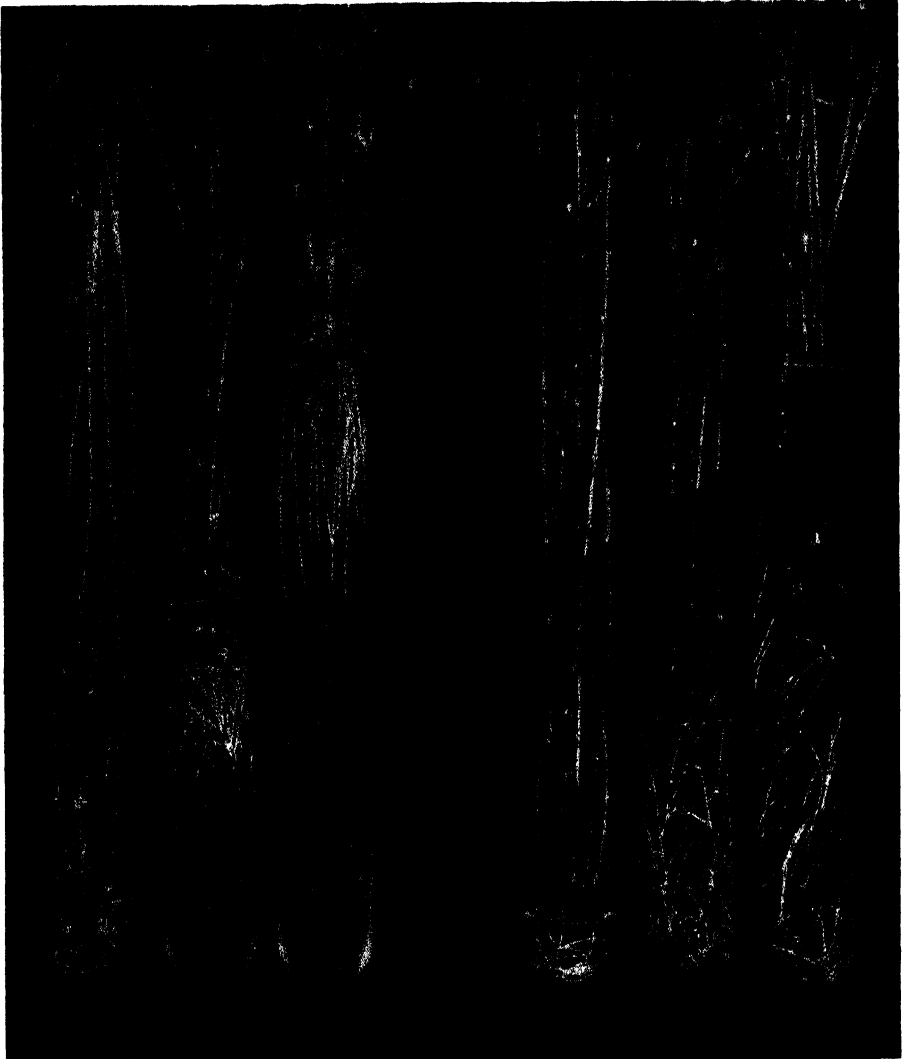


TEXT FIG. 2. *Salvia* cuttings in water from August 2, 1929 to August 24, 1929. *A*, cuttings in tap water not aerated; *B*, cuttings in tap water aerated with oxygen.



TEXT FIG. 3. *Salvia* cuttings in water from August 2, 1929 to August 24, 1929. C, cuttings in water that was boiled to remove the oxygen and then oiled with paraffin oil to decrease oxygen absorption; D, cuttings in water that was boiled, then oiled and aerated.

tendency for roots to form near the surface of non-aerated deep water and at the base of the aerated cuttings. This peculiar response in non-aerated tubes furnished an opportunity to determine the amount of oxygen where roots grew well, or poorly, or not at all. The results of analyses are reported in table I. Text figure 4 shows the root response in different depths of



TEXT FIG. 4. Willow cuttings started October 24, 1929 and photographed November 16, 1929. The glass tubes were 16" long by 2" in diameter. The water depths were 2", 8", and 15".

Left, three tubes not aerated; right, three tubes aerated with oxygen from a commercial cylinder of the gas.

aerated and non-aerated water. Roots grew near the surface of the water in the non-aerated tubes. At the time the experiment was started tap water

had approximately eight parts of oxygen per one million parts of water, but eight days later when roots were starting the oxygen in the non-aerated shallow water had been reduced to 3.2 p.p.m. At the bottom of an 8" column of water the reading was 0.6 p.p.m. and at the bottom of a 15" column no oxygen was detected and no roots had grown. The aerated series showed at the same time rooting and approximately 18 parts of oxygen per million of water at all points in the tubes.

TABLE 1. *Response of Salix pendula (willow) cuttings approximately 18" long taken Oct. 24, 1929 and placed in test tubes (16" × 2") containing three different depths of water to determine the oxygen requirements for root growth*

| Treatment and Date of Analyses | Depth of Water Column, in Inches | Point Below Surface from Which Sample Was Drawn, in Inches | Oxygen p.p.m. | Length of Roots in Inches |
|---|----------------------------------|--|---------------|---------------------------|
| Series A Not aerated Analyses made 11/2 | | | | |
| Tube 1 | 2 | 2 | 3.2 | Starting |
| Tube 2 | 8 | 8 | 0.64 | 0 |
| Tube 3 | 15 | 15 | 0.00 | 0 |
| Series B Aerated with Oxygen Analyses made 11/2 | | | | |
| Tube 1 | 2 | 2 | 18.7 | Starting |
| Tube 2 | 8 | 8 | 19.2 | Starting |
| Tube 3 | 15 | 15 | 18.7 | Starting |
| Series A Not aerated Analyses made 11/6 | | | | |
| Tube 1 | 2 | 1 | 6.6 | 2 |
| Tube 2 | 8 | 1 | 0.5 | $\frac{1}{4}$ |
| Tube 2 | 8 | 8 | 0.1 | 0 |
| Tube 3 | 15 | 1 | 0.6 | $\frac{1}{4}$ |
| Tube 3 | 15 | 7 | 0.3 | 0 |
| Tube 3 | 15 | 15 | 0.2 | 0 |
| Series B * Aerated with Oxygen | | | | About 2 in all depths |

* Analyses were not made throughout on the aerated series because they always showed much more oxygen (approximately 20 p.p.m.) than is required for root growth.

Thirteen days after the experiment had been started, roots were growing near the surface of the water in all the non-aerated tubes. In the shallow water, however, the roots were two inches long with the oxygen at 6.6 p.p.m. as contrasted with one-fourth inch roots in the deep water, where the oxygen had been reduced to 0.6 p.p.m. (see table 1). In the aerated series the roots were approximately two inches in length and the oxygen content was approximately 18 p.p.m. throughout the tubes. Though the roots grew throughout the deep aerated water there was a tendency for the cuttings to exhibit some polarity by having the largest roots near the base. Polarity was disturbed in the deep water, non-aerated series, due to the very low oxygen supply at the base of the cuttings.

TABLE 2. Variation of oxygen supply in water of aerated and non-aerated lots of *Salix pendula* (willow) cuttings. Experiment started 11/22/29

| Treatment and Date of Analyses | Depth of Water Column, in Inches | Point Below Surface from Which Sample Was Taken, in Inches | Oxygen p.p.m. | Length of Roots, in Inches |
|--------------------------------|----------------------------------|--|---|--------------------------------|
| Series C | | | | |
| Not aerated | | | | |
| Analyzed 11/26/29 | | | | |
| Tube 1..... | 3 | 3 | 3.4 | 0 |
| Tube 2..... | 8 | 8 | 1.34 | 0 |
| Tube 3..... | 15 | 15 | 0.64 | 0 |
| Series D | | | | |
| Aerated with oxygen | | | | |
| Analyzed 11/26/29 | | | | |
| Tube 1..... | 3 | 3 | 18.5 | 0 |
| Tube 2..... | 8 | 8 | 18.0 | 0 |
| Tube 3..... | 15 | 15 | 15.0 | 0 |
| Series C | | | | |
| Not aerated | | | | |
| Analyzed 12/6/29 | | | | |
| Tube 1..... | 3 | 3 | 4.8 | $1\frac{1}{4}$ |
| Tube 2..... | 8 | 1 | 0.96 | 0 |
| Tube 2..... | 8 | 8 | 0.64 | 0 |
| Tube 3..... | 15 | 15 | 0.3 | 0 |
| Series D | | | | |
| Aerated with oxygen | | | | |
| Analyzed 12/6/29 | | | | |
| Tube 1..... | 3 | 3 | 19.6 | $1\frac{1}{2}$ |
| Tube 2..... | 8 | 8 | 18.0 | 1 |
| Tube 3..... | 15 | 15 | 18.5 | 1 |
| Series C | | | | |
| Not aerated | | | | |
| Analyzed 12/11/29 | | | | |
| Tube 1..... | 3 | 3 | 5.08 | 1 |
| Tube 2..... | 8 | 4 | 1.44 | $\frac{1}{2}$ |
| Tube 2..... | 8 | 8 | 0.64 | 0 |
| Tube 3..... | 15 | 2 | 0.16 | 0 |
| Tube 3..... | 15 | 15 | 0.2 | 0 |
| Series D * | | | | |
| Aerated with oxygen | | | | |
| | | | All aerated tubes were high in oxygen therefore they were not always tested | Roots in all tubes were 2 to 3 |
| Series C | | | | |
| Not aerated | | | | |
| Analyzed 12/16 to 12/19/29 | | | | |
| Tube 1..... | 3 | 3 | 1.79 | 2+ |
| Tube 2..... | 8 | 1 | 1.18 | 1 |
| Tube 2..... | 8 | 8 | 1.05 | 1 |
| Tube 3..... | 15 | 1 | 0.89 | Roots starting |
| Tube 3..... | 15 | 7 | 0.8 | Roots starting |
| Tube 3..... | 15 | 15 | 0.416 | Roots starting |
| Series D * | | | | |
| Aerated with oxygen | | | | |
| | | | | 3 to 4 |

* Series D omitted because oxygen was high throughout and roots were large (see text fig. 4.)

In another series of experiments with *Salix pendula*, the cuttings in non-aerated water failed to deplete the oxygen supply and as a result roots grew more or less uniformly along the stems from the surface of the water to the base of the cuttings. The oxygen supply varied more than could be accounted for, first running low and then high toward the end of the experiment (see table 2). The tubes not having been wrapped with black paper, there is a possibility that the green stems in light caused an increase of oxygen through photosynthesis. Also, green algae developed in some of the tubes and might have been responsible for keeping the oxygen high.

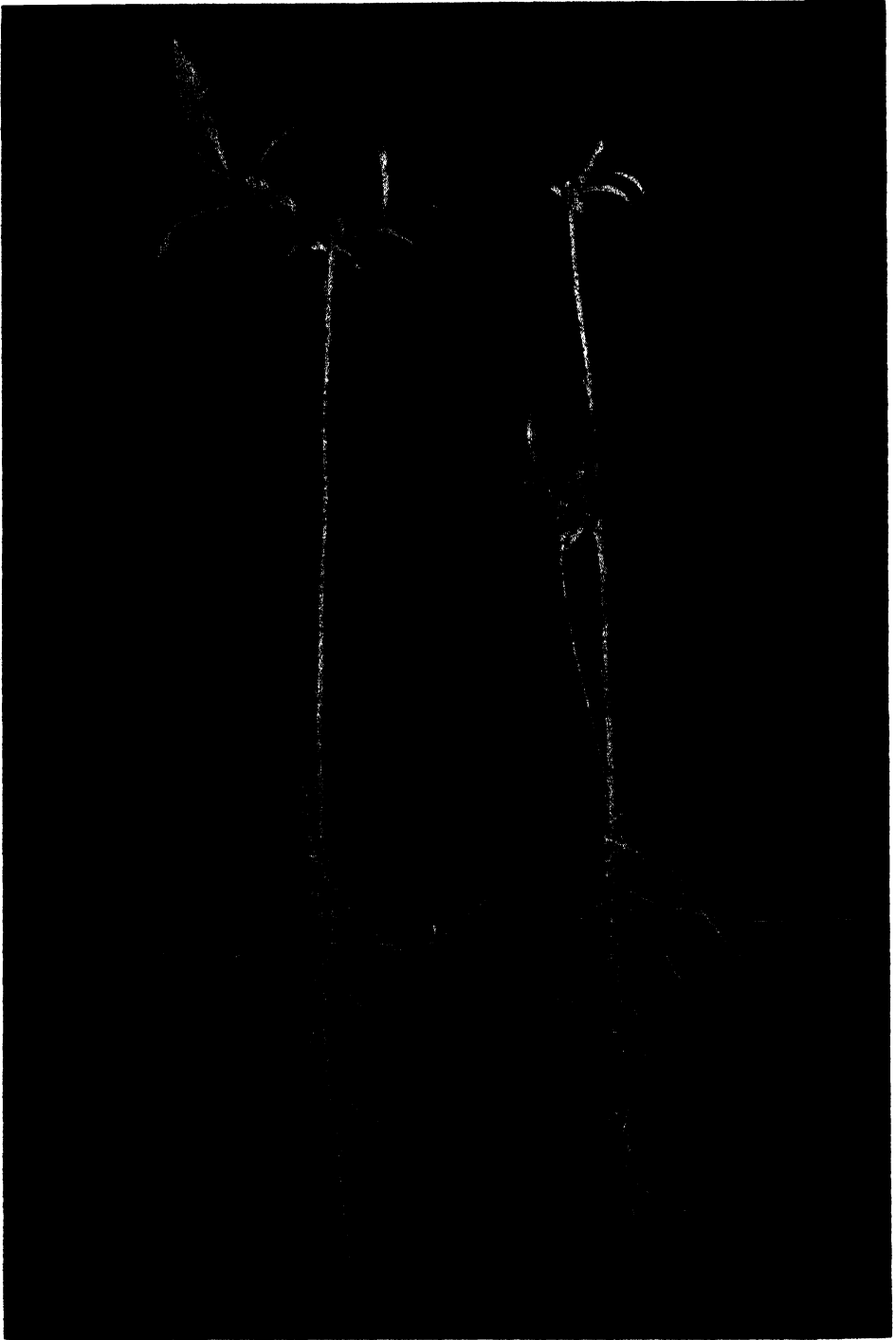
The aerated lot of this series showed two striking differences from the non-aerated. The roots in aerated water were produced near the base of the cuttings and there was very little development of excrescences from the lenticels (text fig. 5). Hahn, Hartley, and Rhodes (9) stated that hypertrophied lenticels were produced on conifer roots in various types of soil in the presence of excessive moisture. Contrary to previous views that such hypertrophies are due to increased sap pressure, these authors believe that such excrescences may also be due to oxygen deficiency. There is a close relationship between the available oxygen supply and the development of hypertrophied lenticels on willow stems. The greatest development yet found was at a place where the supply was approximately one part of oxygen to one million parts of water. The development of these excrescences then diminished toward the base of the cuttings where the oxygen was nearly exhausted (text fig. 5). Aerated willow cuttings produced roots without at the same time producing hypertrophied lenticels, but when the aeration was stopped for a few days excrescences developed rapidly.

Running water through tubes had essentially the same effect on root growth as aerating the water. This result might be expected since the oxygen in tap water was usually eight to ten parts per million of water which is more than has been found necessary for growth of willow roots.

Oxygen requirement varied with the species. *Hedera helix* (English ivy) was grown in aerated and non-aerated water. Table 3 shows the results of

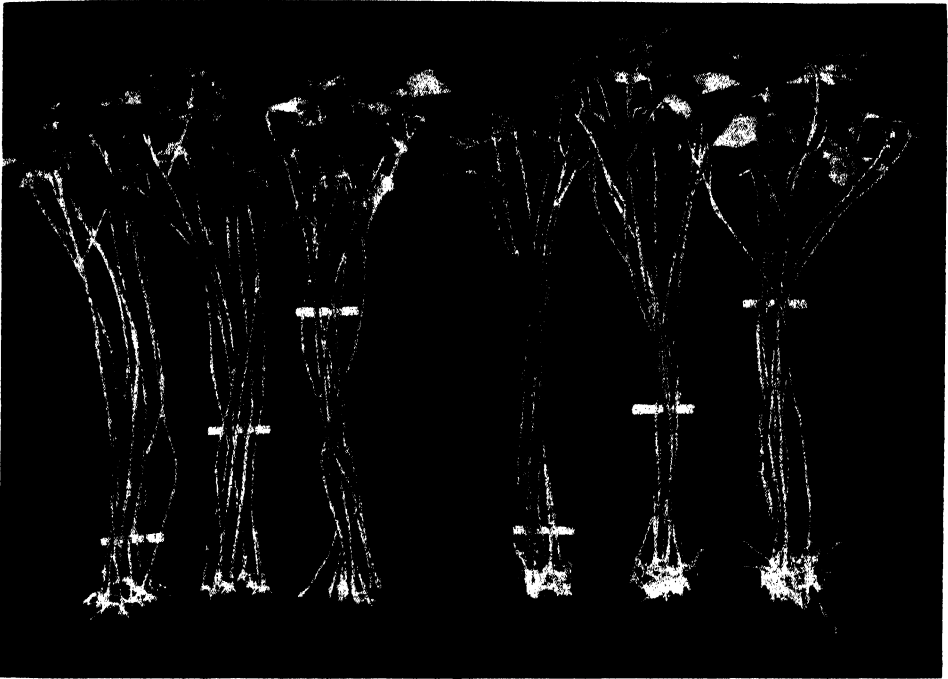
TABLE 3. *Hedera helix* (English ivy) in aerated and non-aerated water. The experiments started Nov. 12, 1929 and the analyses were made Dec. 9, 1929. See text figure 6

| Treatment | Point Below Surface at Which Sample Was Taken, in Inches | Oxygen p.p.m. | Approximate Root Length in Inches |
|---------------------|--|------------------|--------------------------------------|
| Not aerated | | | |
| Tube 1..... | 1½ | 4.5 | 2 |
| Tube 2..... | 4 | 4.0 | 1 |
| Tube 3..... | 7 | 1.8 | ¾ |
| Aerated with oxygen | | | |
| Tube 1..... | 1½ | 22.0 | 2 to 3 |
| Tube 2..... | 4 | 22.4 | 2 to 3½ |
| Tube 3..... | 7 | 20.1 | 2 to 4 |



TEXT FIG. 5. Willow cutting in non-aerated water. Note the hypertrophied lenticels near the surface of the water and how they decrease in size to the base of the cutting where the oxygen supply was low (0.1 to 0.3 parts per million of water).

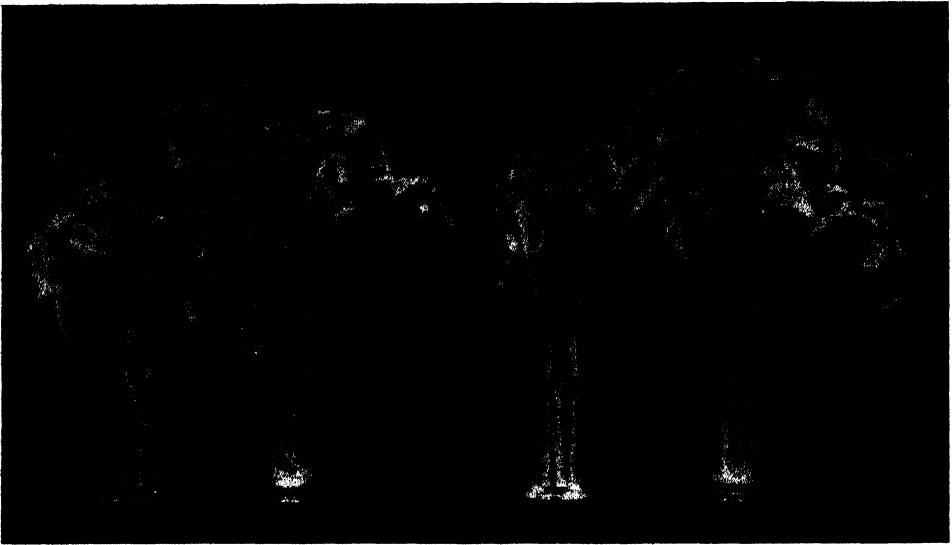
water analyses 30 days after the experiment was started, and text figure 6 shows the appearance of the roots. The stems of the cuttings were green and it is likely that through the photosynthetic process the oxygen content



TEXT FIG. 6. Aerated and non-aerated English ivy cuttings in three depths of water. The white line across the stems indicates the height of the water column. The shallow cuttings were in one and one-half inches of water, the medium four inches, and the deep seven inches. The experiment started November 12, 1929 and the photograph was made December 13, 1929, a total of 32 days. Left, not aerated; right, aerated.

was kept higher in the non-aerated tubes than would have been true for darkened cylinders. But even though the oxygen was as high as four parts per million in the non-aerated four-inch column of water the roots were much inferior to those of similar cuttings in aerated water. The specimens in seven inches of non-aerated water produced roots only one-eighth of an inch in length though the oxygen was 1.8 parts per million, which would be sufficient for good root growth of willow cuttings. The results of this experiment indicate that the rate of growth of ivy roots varies within limits according to the oxygen supply. With 1.8 p.p.m. of oxygen they made one-eighth of an inch of growth in contrast to three to four inches of growth in aerated water where the oxygen was 20 p.p.m. It is not likely that ivy would be benefited from supplying more than ten parts of oxygen per million of water where other factors are not limiting. The difference between the root growth of the two shallow groups is only slight while the oxygen content ranges from 4.5 p.p.m. in the non-aerated to 20.1 p.p.m. in the aerated.

The oxygen supply in non-aerated water in which *Lycopersicum esculentum* (tomato) cuttings were kept varied with the depth of the water and also with exposure to light. Since the oxygen was never depleted in the deep water tubes, it is likely that the green tissue gave off oxygen through photosynthesis, thus aerating the water. Table 4 shows that several sets of tomato cuttings which had black paper wrapped around the containers nearly exhausted the oxygen supply whereas those that were not wrapped had four to six parts of oxygen per million of water throughout the time the experiment continued. The stems of tomato cuttings in deep water disintegrated readily when the container was wrapped and not aerated (text fig. 7).



TEXT FIG. 7. Tomato cuttings in 8 inches of water to find combined effect light and aeration *vs.* dark and aeration. Left to right (1) control in light, (2) aerated with oxygen in light, (3) control where the cylinder was wrapped with black paper, (4) cylinder wrapped with black paper and then aerated.

Note that no. 1 though rooting near the surface of the water instead of at the base, is in good condition while no. 3 treated like no. 1 except that cylinder was wrapped with black paper, is in bad condition. The cause of deterioration was due to a deficiency in oxygen.

Table 5 gives further proof that the green stems in light have an aerating effect on the water. When the water was oiled to decrease oxygen absorption from the air and wrapped to shut out the light, the final analysis showed the oxygen to be only 0.48 p.p.m., as compared to 6.7 p.p.m. for the check. The table further shows that oil decreased the oxygen absorption from the air. The oiled water in light contained 3.2 p.p.m. as contrasted with 6.7 p.p.m. for the check where the water had not been oiled.

Callus formation varied with depth of the tissue under water. Dorothy Perkins rose cuttings formed both roots and callus in three inches of water

TABLE 4. *The results of analyses to show the amount of oxygen in water in which tomato cuttings were grown. The purpose of the experiment was to determine whether light had any effect on the oxygen content of the water in which the green stems were immersed. Each set contained three tomato cuttings in a large glass tube filled with water*

| Treatment | Set No. | Date Started | Date Analyzed | Point Below Surface at Which Sample Was Taken, in Inches | Oxygen p.p.m. | Root Length, in Inches |
|-----------------------------------|---------|--------------|---------------|--|---------------|------------------------|
| In direct light in the greenhouse | 1 | 11-22 | 11-25 | 14 | 4.28 | 0 |
| | 2 | 11-22 | 11-25 | 8 | 6.2 | starting |
| | 1 | 11-22 | 11-26 | 14 | 5.5 | starting |
| | 2 | 11-22 | 11-26 | 8 | 6.4 | $\frac{1}{8}$ |
| | 1 | 11-22 | 11-27 | 14 | 5.12 | $\frac{1}{2}$ |
| | 2 | 11-22 | 11-27 | 8 | 6.2 | $\frac{1}{2}$ |
| | 1 | 11-22 | 11-29 | 14 | 6.56 | 1 to 2* |
| | 2 | 11-22 | 11-29 | 8 | 4.32 | 1 to 1 $\frac{1}{2}$ |
| | 5 | 11-29 | 12-2 | 9 | 4.35† | 0 |
| | 5 | 11-29 | 12-3 | 9 | 6.78 | $\frac{1}{4}$ |
| | 5 | 11-29 | 12-4 | 9 | 6.72 | $\frac{3}{4}$ |
| | 7 | 12-4 | 12-9 | 2 | 4.12 | 1 |
| | 8 | 12-4 | 12-9 | 2 | 5.44 | 1 $\frac{1}{2}$ |
| Tubes covered with black paper | 3 | 11-22 | 11-25 | 14 | 1.41 | 0 |
| | 4 | 11-22 | 11-25 | 8 | 3.84 | starting |
| | 3 | 11-22 | 11-26 | 14 | 1.47 | 0 |
| | 4 | 11-22 | 11-26 | 8 | 4.8 | $\frac{1}{8}$ |
| | 3 | 11-22 | 11-27 | 14 | 0.57 | 0 |
| | 4 | 11-22 | 11-27 | 8 | 2.36 | $\frac{1}{2}$ |
| | 3 | 11-22 | 11-29 | 14 | 0.7 | 0 |
| | 4 | 11-22 | 11-29 | 8 | 2.04 | 1 to 2* |
| | 6 | 11-29 | 12-2 | 9 | 1.2 | 0 |
| | 6 | 11-29 | 12-3 | 9 | 2.84 | 0 |
| | 6 | 11-29 | 12-4 | 9 | 3.2 | roots starting |
| | 8 | 12-4 | 12-9 | 2 | 1.7 | 1 $\frac{1}{2}$ |
| | 9 | 12-4 | 12-9 | 2 | 2.6 | 1 $\frac{1}{2}$ |

* Roots in aerated water were 3" to 4" in length.

† Cloudy day.

TABLE 5. *Oxygen content of water in which tomato cuttings were growing. The purpose of the experiment was to determine the effect of light on the oxygen supply of the water in which green stems were kept. The method was the same as described for Table 4, except that in some cases paraffin oil was placed on the surface of the water to decrease oxygen absorption from the air. The water samples analyzed were taken from approximately 9" below the surface*

| Treatment | Set No. | Date Started | Date Analyzed | Oxygen p.p.m. |
|---|---------|--------------|---------------|---------------|
| Check in light..... | 1 | 11-29 | 12-2 | 4.35* |
| | 1 | 11-29 | 12-3 | 6.78 |
| | 1 | 11-29 | 12-4 | 6.72 |
| Oiled but in light..... | 2 | 11-29 | 12-2 | 4.03 |
| | 2 | 11-29 | 12-3 | 4.8 |
| | 2 | 11-29 | 12-4 | 3.2 |
| Tubes wrapped with black paper..... | 3 | 11-29 | 12-2 | 1.2 |
| | 3 | 11-29 | 12-3 | 2.8 |
| | 3 | 11-29 | 12-4 | 3.2 |
| Oiled and wrapped with black paper..... | 4 | 11-29 | 12-2 | 0.9 |
| | 4 | 11-29 | 12-3 | 0.38 |
| | 4 | 11-29 | 12-4 | 0.48 |
| Oiled and aerated with oxygen..... | 5 | 11-29 | 12-2 | 23.0 |
| | 5 | 11-29 | 12-3 | 24.0 |
| | 5 | 11-29 | 12-4 | 20.0 |

* Cloudy day

but not in 8 inches of water. No analyses were made but it seems fair to assume that there were about 4 or 5 p.p.m. of oxygen in the shallow water and much less in the deep water.

Of the oxidizing agents, hydrogen peroxid and potassium permanganate have been most effective. Text figure 8 shows the effect on production of roots of different concentrations of hydrogen peroxid in tap water. Evidently the upper limits had not been reached with three cubic centimeters of hydrogen peroxid per week. The analysis indicated as high as 17 parts of oxygen per million of water where three cubic centimeters per week had been used, but the supply varied from the time the new supply was added, becoming low by the end of the week. As shown by the photograph (text fig. 8) the place where roots grew differed greatly from that of aerated cuttings (text fig. 4). In aerated water, the roots grew best at the base but in water containing hydrogen peroxid the best roots grew near the surface of the water. This response was probably due to the low supply of oxygen maintained by the hydrogen peroxid toward the end of the period. Analyses of the water shortly after the hydrogen peroxid was added indicated oxygen as high as 50 p.p.m., but after six days the supply was about that of the control tubes.



TEXT FIG. 8. Three sets of willow cuttings showing the effects of three different concentrations of hydrogen peroxid in tap water. The experiment was started December 21, 1929, and the results photographed on January 1, 1930.

Left, tube given one cc. of hydrogen peroxid each week.

Middle, tube given two cc. of hydrogen peroxid each week.

Right, tube given three cc. of hydrogen peroxid each week.

Roots from willow cuttings were produced along the entire length of stems which were immersed in weak solutions of potassium permanganate. Cuttings so treated differed from those in water aerated with oxygen in that those aerated produced most roots at the basal end of the stems, and a decreasing number toward the surface of the water. Polarity was not so much in evidence where permanganate was substituted for aeration. Quantitative measurements for free oxygen in the permanganate solution were not made because the permanganate interferes with the operation of one of the reagents (manganese sulfate) of the Winkler method. It is not clear just how an oxidizing agent like permanganate can substitute for the oxygen requirements of cuttings in water. Curtis, (6, p. 97) states that it is generally well known that when potassium permanganate comes in contact with organic matter, manganese dioxid is precipitated and oxygen is liberated. There is some doubt as to whether free molecular oxygen is actually liberated in the water, but however that may be, permanganate in some way partially substitutes for aeration. In the case of hydrogen peroxid probably free oxygen is actually liberated in the water.

DISCUSSION

Several striking responses have occurred in the course of these experiments. *Salvia* cuttings commonly show strong polarity and have no tendency to root up along the stem if they are properly aerated, but if the oxygen content of the water is lower than the minimum required for this species then roots tend to grow near the surface of the water. The oxygen content decreases from the surface down to the bottom of the water column thus accounting for poor root growth at the base of the stems. If a large number of cuttings are placed in a small volume of deep water, they soon deplete it of oxygen and then show signs of wilting. Livingston and Free (10) found that coleus and heliotrope growing in soil would wilt readily if completely deprived of oxygen. *Salvia* cuttings wilted even though the stems were immersed in 15 inches of water. Aerated cuttings in deep water remained in good condition and formed roots at the basal end of the stems. Long tomato cuttings in deep water responded like *salvia*, and when in darkness the non-aerated stems disintegrated very readily. Even wrapping of the cylinders with black paper caused the cutting to wilt and the stems to decompose. A thin layer of paraffin oil on the water decreased the absorption of oxygen from the air and hastened the destruction of cuttings in wrapped cylinders. The green stems partially substituted for aeration when the light intensity was great enough. This was accomplished, presumably, through photosynthesis by the green stems under water. Bergman (2) noted that the oxygen content of water containing submerged cranberry plants was greater on clear than on cloudy days.

Aerated cuttings produced their best roots near the basal end of the stems while the roots of non-aerated cuttings were best near the surface of the

water. Even willow stems, which have the capacity to root along the stem, showed strong polarity if placed in aerated water. When the basal end of a stem had an inadequate oxygen supply, the functioning region moved up near the surface of the water where the oxygen content was highest. The basal end remained alive and resumed normal growth when properly aerated. This suggests that to maintain a correlation influence the cells must not only be alive but also have the conditions which permit of growth.

Hypertrophied lenticels were in some way associated with aeration. They were largest in willow stems that were submerged in non-aerated water. Aerated water inhibited development of these excrescences. In non-aerated water they were largest where the roots were best and decreased in size together with the roots down toward the base of the stem where the oxygen content was nearly depleted. Like the roots in non-aerated water, hypertrophied lenticels grew well when the oxygen was 1 to 2 p.p.m., but unlike the roots they did not grow well in aerated water where the oxygen was 20 p.p.m. This suggests that tissues within a stem may not all be equally affected by a given oxygen supply. Hahn, Hartley, and Rhodes (9) noted that hypertrophied lenticels of conifer roots were produced in the presence of excessive moisture but suspected that they might be related to a deficiency of oxygen. Since cuttings immersed in water high in oxygen do not produce hypertrophied lenticels, water alone is not the factor which induces this growth. Water with a low oxygen content (1 to 2 p.p.m.), however, stimulates the cells which produce hypertrophies. Two sets of tissues within a single stem have, therefore, two different oxygen requirements for growth.

The cuttings from different species tested show different oxygen requirements as reported by various authors for growth of seedlings in soil. Tomato, salvia, and ivy have a high oxygen requirement while willow roots can grow when the oxygen is only 1 p.p.m. When enough oxygen is available to permit of normal growth, the supplying of more oxygen does not cause increased growth.

SUMMARY

1. *Salvia* cuttings formed roots at the base of the stems that were placed in two inches of water. Similar cuttings in five-inch depths of water had a tendency to root along the stems toward the surface of the water. *Salvia* cuttings in water seven inches deep were either slow to respond or did not root at all.

2. *Salvia* cuttings aerated with oxygen produced basal roots in shallow and deep water alike.

3. A thin film of paraffin oil on the surface of the water prevents rooting by interfering with absorption of oxygen from the air.

4. *Salvia* cuttings in water covered with a thin film of paraffin oil formed roots readily when the water was aerated with oxygen.

5. *Salix pendula* cuttings formed roots at the base of the stems when in shallow water, but when placed in deep water (eight inches or more) roots

formed on the stems near the surface of the water. Cuttings in aerated water formed roots at the base of the stems and practically none at the surface of the water. A deficiency of oxygen at the bottom of a deep water column disturbed the natural polarity of willow cuttings.

6. Analyses indicate that willow cuttings will form roots in water if the oxygen is 1 p.p.m. or more, this being the amount frequently found at the surface of a deep water column where roots were growing. Practically no oxygen could be found at the bottom of a 15 inch column of water in which ten cuttings were growing. Analyses of aerated water showed 20 to 30 p.p.m. at all depths.

7. Hypertrophied lenticels were produced more abundantly in non-aerated water than in aerated water. As with roots, excrescences varied from the surface of the water, becoming less and less toward the bottom of the column where the oxygen was practically depleted.

8. Running water was in effect equal to aeration due to the fact that the normal oxygen content of tap water was higher than the optimum amount required by the different species used.

9. English ivy required a higher oxygen supply for root growth than willow. Where the oxygen was 4 p.p.m. the root growth was less than where the supply was 20 p.p.m.

10. The oxygen supply in non-aerated water surrounding tomato cuttings varied with the depth of the water column and also with exposure to light. Cuttings in cylinders wrapped with black paper soon depleted the water of oxygen while those in cylinders exposed to light aerated the water through photosynthesis.

11. Tomato cuttings in deep water disintegrate readily if the cylinders containing the water are wrapped with black paper or the whole lot is kept in a dark room.

12. Callus forms on Dorothy Perkins rose cuttings in shallow water but not in deep water.

13. Oxidizing agents such as hydrogen peroxid and potassium permanganate increased the amount of rooting but they were not so effective as aeration.

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MEIOSIS OF MICROSPOROGENESIS IN THE JUGLANDACEAE

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A study of the cytological conditions in the Betulaceae (Woodworth 7, 8, 9, 10) has proved so productive that it was thought worth while to investigate conditions in the Juglandaceae, which is considered by some to be a closely related family.

MATERIALS AND METHODS

Material for this study was gathered from labelled specimens in the Arnold Arboretum. Staminate catkins were collected in the early afternoon on warm days of early May. Carnoy's fluid was used for killing and fixing. The catkins were imbedded in pyroxylin, an improved nitrocellulose. Heidenhain's iron haematoxylin was the only stain used. A Zeiss microscope equipped with a 140° Abbe condenser, a 3-mm. 140° apochromatic objective, and a 20 × compensating ocular was used for study and drawing. A Spencer camera lucida was employed for outlining the illustrations. The figures in the plates are magnified 2000 times except figure 13, which is 350 times. The chromosomes, averaging about 1 micron in diameter, are so small that a study of their structure is quite impossible. *X* signifies the haploid number of chromosomes.

JUGLANS L., THE WALNUT

All species of this genus investigated have thirty-two chromosomes in the sporophytic tissue and sixteen in the gametophytic tissue.

Juglans regia L. English Walnut. *X*-16. Plate I, figure 1 is a drawing of the homotypic metaphase with one spindle in side view and the other in polar view, the latter showing the sixteen chromosomes. The meiotic phenomena are regular, resulting in the normal tetrads of microspores. This plant hybridizes with *J. Hindsii*, *J. nigra*, *J. intermedia*, *J. cinerea*, and *J. Sieboldiana* (Rehder, 3), the latter being considered in this paper under *J. notha*.

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Juglans rupestris Engelm. X-16. Figure 2 pictures a metaphase plate of the heterotypic division with the sixteen chromosomes. Meiosis is typically normal.

Juglans nigra L. Black Walnut. X-16. A cell in the heterotypic metaphase is shown in polar view in figure 3. Although the sixteen chromosomes are extremely small they are quite distinct from one another and are easily counted in any of the active phases of microsporogenesis. This plant hybridizes with *J. regia* and *J. Hindsii*.

Juglans cinerea L. Butternut. X-16. The reduction division is normal. The sixteen chromosomes are seen in the polar view of the heterotypic metaphase in figure 4. This species crosses with *J. regia* and *J. Sieboldiana*.

Juglans mandshurica Maxim. Manchurian Walnut. X-16. Figure 5 illustrates the metaphase plate of the first division with sixteen chromosomes. Meiosis is quite regular, producing normal tetrads of microspores.

Juglans Sieboldiana var. *cordiformis* Mak. X-16. A plate of the heterotypic metaphase is shown in figure 6. Sixteen chromosomes are clearly demonstrated in all active phases of meiosis. Rehder writes (3) that *J. Allardiana*, *J. coarctata*, *J. Lavalleyi*, and *J. subcordiformis*, all of Dode, really belong to *J. Sieboldiana*, being intermediates between the type and the var. *cordiformis*. *J. Sieboldiana* crossed with *J. regia* produces *J. notha* which is treated below; it also hybridizes with *J. cinerea* (Rehder, 3).

The reduction division in the above six species is marked by regularity. The microspores are produced in normal tetrads and the pollen grains are uniform in their morphological perfection. The cytological conditions which obtain in microsporogenesis of *J. notha*, the next plant to be considered, are different, being very irregular and producing abnormal microspores. This plant is a known hybrid which originated in 1878 (Rehder, 3).

It is important to note here that the research material for this study was collected on the same day, during the same hour, in the same killing and fixing fluid, and that these catkins were imbedded at the same time and with identical technique as that used for the materials of the six species described above. As a further check, duplicate materials were studied in two successive years. Both collections showed identical conditions for all species. The writer takes this opportunity to stress these facts to avoid possible criticism on the grounds of the disorganizing effects of cold, killing and fixing agents, and cytological technique on the reduction division. In making an examination of meiosis in a particular family or genus it is usually found that the majority of the members are normal; indeed this is wholly to be expected. If technique produces abnormalities one would hardly expect to find the greater part of the material to be normal.

Juglans notha Rehd. (*J. Sieboldiana* × *regia*). X-16. The plant studied is a magnificent tree in the Arnold Arboretum. It is known to be of heterozygous origin (Rehder, 3).

Microsporogenesis is abnormal in the manner typical of so many known hybrid plants (Sharp, 5). During diakinesis the chromosomes for the most part pair in the normal fashion. At the metaphase, however, these pairs do not become arranged in an equatorial plate. They are scattered up and down the spindle and several of the bivalent chromosomes separate without passing to the middle region of the spindle (fig. 7). Thus the anaphase is initiated unusually early with the omission of the metaphase. Figure 8 shows the typical condition of the early anaphase, most of the gemini having divided with some of the chromosomes already at the poles. The late anaphase is pictured in figure 9, many of the chromosomes lagging on the spindle. Although the second division is not necessarily expected to be affected by incompatible chromosome groups, it is nevertheless marked by irregularities in this plant.

Figure 10 illustrates the condition of the spindle at the time of the homotypic metaphase. Here again no distinct equatorial plate is formed. The anaphase of this division again shows (fig. 11) the chromosomes lagging all along the spindle. Occasionally some of the chromosomes which lag during the heterotypic anaphase (fig. 9) do not progress to the poles, and therefore they are not incorporated in the polar nuclei. These bodies may form an extra nucleus. Figure 12 shows a cell in which this has happened; the extra nucleus, as well as the usual two nuclei, is in the anaphase.

In figure 13 are seen a group of microspores typical of any maturing anther in the catkins of this hybrid. The irregularities and abnormalities of meiosis cause a wholesale malformation of microspores. There are some giant grains which must be the product of a whole mother cell. Others are of average size but are shrunken and highly vacuolate. Still others are tiny grains (microcytes) which are formed as a result of the extrusion of chromosomes into the cytoplasm. Practically all of these spores appear to be sterile.

Cytomixis is a common occurrence during all phases of the heterotypic division. Frequently groups of chromosomes are seen to be migrating from one cell to another over these cytoplasmic connections.

CARYA NUTT., THE HICKORY

The species of *Carya* investigated are either diploid with thirty-two chromosomes or tetraploid with sixty-four chromosomes in the sporophytic tissue.

Carya ovata K. Koch. Shagbark Hickory. X-16. A polar view of a heterotypic metaphase plate with its sixteen chromosomes is seen in Plate LI, figure 14. Meiosis is quite regular. This species crosses with *C. cordiformis*, producing *C. Laneyi*, a variety of which is treated below. This species is the best nut producer next to *C. pecan* and there are several named varieties in cultivation.

Carya laciniosa Loud. Big Shellbark Hickory, Kingnut. X-16. All phases of the meiotic division show the sixteen chromosomes. Figure 15 is the heterotypic metaphase plate. Meiosis is normal. This species hybridizes with *C. pecan* (Rehder, 3).

Carya alba K. Koch. Mockernut. X-32. This species is tetraploid. The thirty-two chromosomes are seen in the equatorial plate of the first division in figure 16. Meiosis is regular. Occasionally in the maturing anthers diads and monads are seen among the smaller grains of the tetrads. No evidence for the cause of this has yet been noted. If the diads contain sixty-four chromosomes and the monads contain one hundred twenty-eight their success in fertilization might produce highly polyploid offspring. This species hybridizes with *C. pecan*.

Carya glabra Sweet. Pignut. X-32. This species is also tetraploid. Figure 17 is a polar view of the heterotypic metaphase with thirty-two chromosomes. Meiosis normal.

Carya ovalis Sarg. Small Pignut. X-32. Figure 18 is the equatorial plate of the first division with thirty-two chromosomes. This is the third tetraploid species. Meiosis normal. Sargent has described five varieties under this species (Rehder, 3).

Carya cordiformis K. Koch. Bitternut. X-16. Meiosis is quite normal. The metaphase plate of the first division pictured in figure 19 shows the sixteen chromosomes. This species crosses with *C. pecan*.

Carya Laneyi var. *chateaugayensis* Sarg. X-16. (*C. ovata* × *cordiformis* = *C. Laneyi*.) This variety resembles *C. cordiformis* more than does the type species-hybrid. Meiosis is not normal. The sixteen chromosomes in the mother cells of some anthers behave quite regularly but in others they are very tardy in their movement on the spindle, thus exhibiting the typical lagging seen in other hybrid plants. Figure 20 pictures a heterotypic met-anaphase where several of the bivalent chromosomes are pulling apart, the formation of an equatorial plate having been omitted. The chromosomes lag throughout the phases of the first and second divisions, these irregularities apparently being the cause of the sterility of a small percentage of the pollen.

PTEROCARYA KUNTH., THE WING NUT

Pterocarya Rehderiana Schneid. (*P. fraxinifolia* × *stenoptera*). X-16. This hybrid is more vigorous and hardier than either of the parents. Several magnificent specimens in the Arnold Arboretum are in excellent condition while both of the parents have long since died out, the climate of this latitude being too severe for them. This hybrid spreads by suckers.

Meiosis is irregular. Figure 21 pictures the heterotypic metaphase plate with sixteen chromosomes. Figure 22 is another cell in the same phase with but fifteen chromosomes, which condition is frequently found. At interkinesis bits of chromatin are occasionally seen on the spindle while

some of them are in the cytoplasm (fig. 23). Counts of the chromosome complement at homotypic metaphase usually show sixteen as in the left plate of figure 24, while sometimes only fifteen are present as in the plate to the right in the same figure, although in this case the sixteenth chromosome is seen in the cytoplasm near by. For the most part tetrad formation results in microspores which look to be normal.

Cytomyxis is often noted during meiosis. Figure 25 pictures two cells during the heterotypic division connected by cytoplasmic strands. The lower cell has lost all but three of its chromosome complement and it appears that the other thirteen migrated over a spindle-like structure to the upper cell. Figure 26 shows the same type of thing at homotypic telophase. Some workers hold that this is caused by the fixative (Rosenberg, 4; Sinoto, 6). If this is the case we would expect the same type of phenomenon to appear in the species of *Juglans* and *Carya* which were treated with the same reagents. It does not. If this cytomyxis is due to the instability of a heterozygous protoplasm, this is then the ultimate cause of the cytomyxis rather than the fixative. Church (1) has considered this subject. Cytomyxis may account for the occasional loss of a chromosome as noted above.

PHYLOGENY

The reports in previous papers (Woodworth, 7, 8, 10) show that in the light of chromosome numbers there are two definite sections in the Betulaceae; one, including *Betula*, *Alnus*, and *Corylus*, with fourteen as the fundamental number of chromosomes, running up to octoploid forms with eighty-four chromosomes; and the other section including *Carpinus*, *Ostrya*, and *Ostryopsis*, with eight as the fundamental number, also containing an octoploid representative. Such a grouping of betulaceous genera into two families is further accentuated by the fact that *Betula*, *Alnus*, and *Corylus* complete meiosis and microspore formation in the fall previous to the flowering season, while *Carpinus*, *Ostrya*, and *Ostryopsis* undergo meiosis during the early weeks of the flowering season. The above data would add weight to Hutchinson's (2) subdivision of the Betulaceae into two families, the Betulaceae and the Corylaceae, except that *Corylus* itself should be in the first family, which would then necessitate a new name for the second family.

These chromosome numbers are mentioned above because the Juglandaceae, from this study, have been found to have sixteen as the fundamental number of chromosomes. Although Hutchinson (2) entirely divorces the Juglandaceae from the Amentiferae the intimation is at hand that the walnut family may be phylogenetically related to the eight-chromosome (*Carpinus*, *Ostrya*, *Ostryopsis*) branch of the Betulaceae. This suggestion is made here with the thought that it may prove of some value in untangling the phylogeny of the Amentiferae.

SUMMARY

1. *Juglans regia*, *J. rupestris*, *J. nigra*, *J. cinerea*, *J. mandshurica*, *J. Sieboldiana* var. *cordiformis*, all have sixteen as the haploid number of chromosomes and all show normal meiosis.

2. *Juglans notha* is a known hybrid. It shows the irregularities of meiosis characteristic of heterozygous plants. It is emphasized that these abnormalities are natural, not being due to the technique employed. Cytomyxis is seen to be accompanied by chromosome migration.

3. *Carya ovata*, *C. laciniosa*, *C. cordiformis*, have sixteen as the haploid complement and exhibit regular meiosis.

4. *Carya alba*, *C. glabra*, *C. ovalis*, are tetraploid species with thirty-two chromosomes as the haploid number. Meiosis is normal.

5. *Carya Laneyi* var. *chateaugayensis* is a known hybrid. It is diploid as are both its parents. Meiosis is abnormal as in other hybrids.

6. *Pterocarya Rehderiana* is a known hybrid. It has sixteen haploid chromosomes. Meiosis is somewhat abnormal due to its heterozygous nature. Cytomyxis is accompanied by chromosome migration.

7. *Betula*, *Alnus*, and *Corylus* with fourteen chromosomes, and *Carpinus*, *Ostrya*, and *Ostryopsis* with eight chromosomes as the respective fundamental numbers form two rather distinct groups in the Betulaceae. The Juglandaceae with sixteen chromosomes may have a phylogenetic connection with the second of the above groups.

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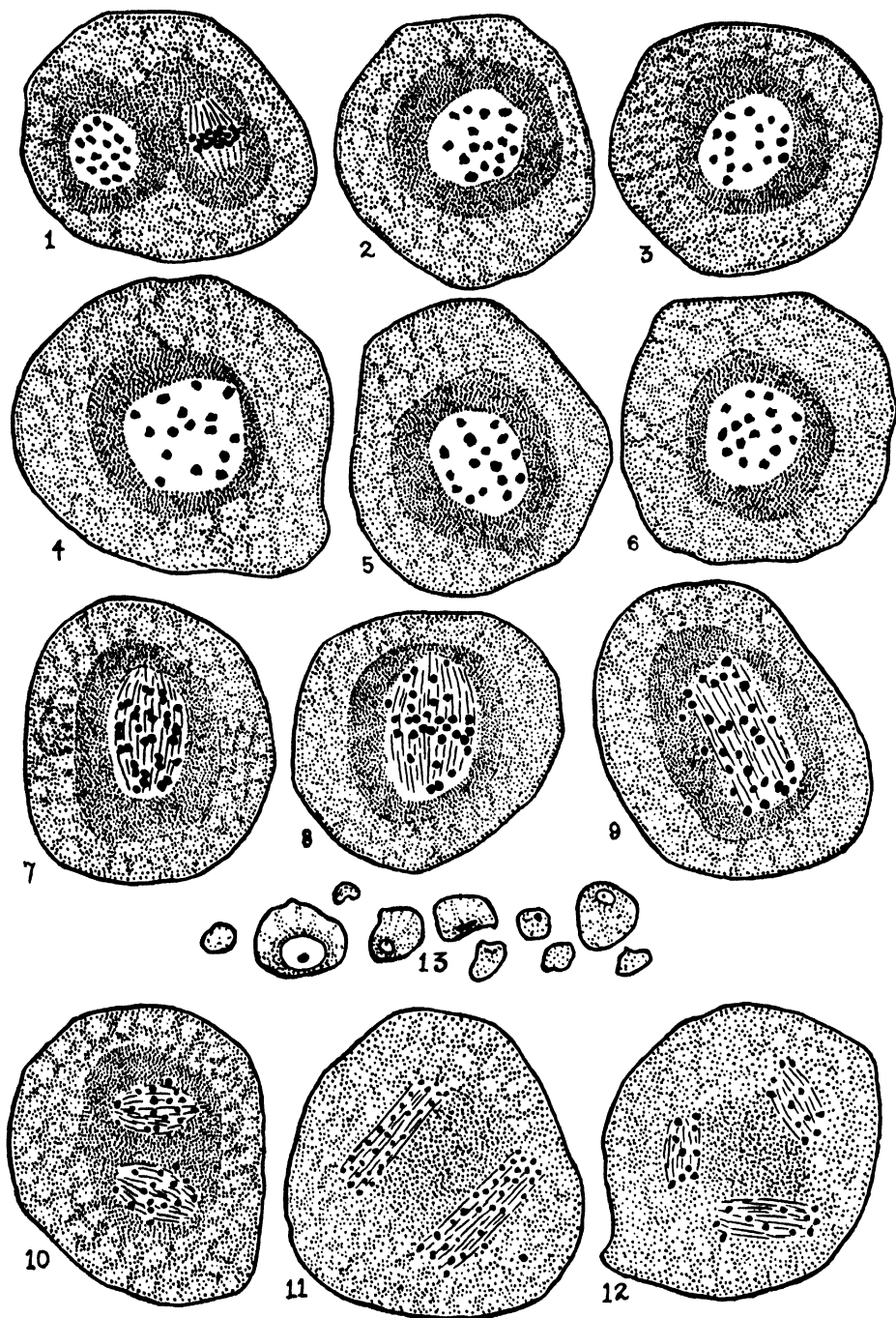
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EXPLANATION OF PLATES

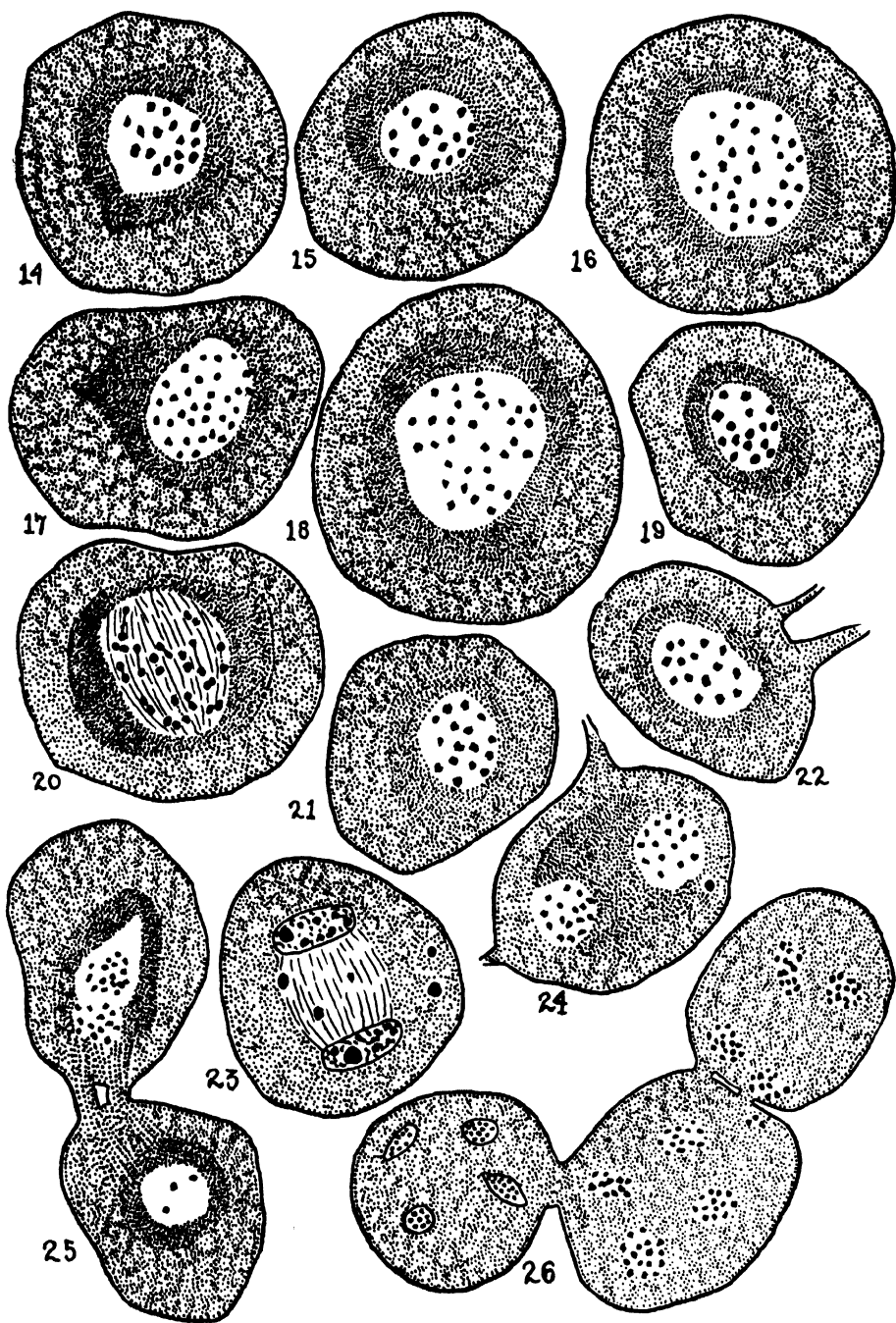
PLATE L

FIG. 1. *Juglans regia*, homotypic metaphase.

FIG. 2. *J. rupestris*, heterotypic metaphase plate.



WOODWORTH: MICROSPOROGENESIS IN JUGLANDACEAE



WOODWORTH: MICROSPOROGENESIS IN JUGLANDACEAE

- FIG. 3. *J. nigra*, heterotypic metaphase plate.
FIG. 4. *J. cinerea*, heterotypic metaphase plate.
FIG. 5. *J. mandshurica*, heterotypic metaphase plate.
FIG. 6. *J. Sieboldiana* var. *cordiformis*, heterotypic metaphase plate.
FIG. 7. *J. notha* (*J. Sieboldiana* \times *regia*), early heterotypic, corresponds to metaphase.
FIG. 8. *J. notha*, early anaphase of heterotypic.
FIG. 9. *J. notha*, late anaphase of heterotypic.
FIG. 10. *J. notha*, early homotypic, corresponds to metaphase.
FIG. 11. *J. notha*, homotypic anaphase.
FIG. 12. *J. notha*, homotypic anaphase with three spindles.
FIG. 13. *J. notha*, microspores; giant grains, shrunken grains and microcytes. $\times 350$.

PLATE LI

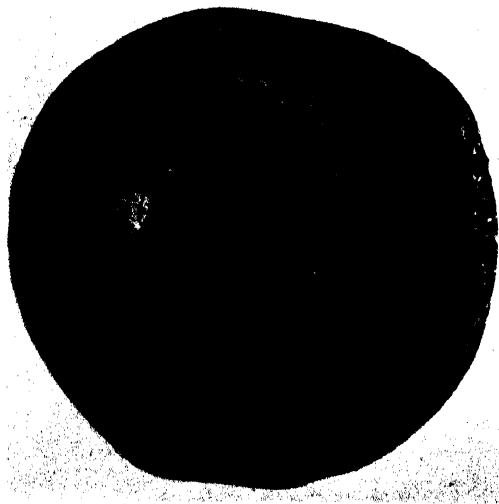
- FIG. 14. *Carya ovata*, heterotypic metaphase plate.
FIG. 15. *C. laciniosa*, heterotypic metaphase plate.
FIG. 16. *C. alba*, heterotypic metaphase plate.
FIG. 17. *C. glabra*, heterotypic metaphase plate.
FIG. 18. *C. ovalis*, heterotypic metaphase plate.
FIG. 19. *C. cordiformis*, heterotypic metaphase plate.
FIG. 20. *C. Laneyi* var. *chateaugayensis* (*C. Laneyi* = *C. ovata* \times *cordiformis*), heterotypic meta-anaphase.
FIG. 21. *Pterocarya Rehderiana* (*P. fraxinifolia* \times *stenoptera*), heterotypic metaphase plate, sixteen chromosomes.
FIG. 22. *P. Rehderiana*, same with fifteen chromosomes.
FIG. 23. *P. Rehderiana*, interkinesis.
FIG. 24. *P. Rehderiana*, homotypic metaphase.
FIG. 25. *P. Rehderiana*, cytomyxis at heterotypic metaphase.
FIG. 26. *P. Rehderiana*, cytomyxis at homotypic anaphase.

THE RESPONSE TO ULTRA-VIOLET IRRADIATION SHOWN BY VARIOUS RACES OF *GLOMERELLA CINGULATA*

F. L. STEVENS

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Some time ago I published two papers (1928*a*, 1928*b*) showing that ultra-violet irradiation of colonies of certain strains of *Glomerella cingulata* (Stoneman) Spaulding and von Schrenk, in very small dosages, resulted immediately in the production of immense numbers of perithecia in areas where none were produced without such irradiation; and also that in *Coniothyrium* the production of pycnidia was in a similar way effected.



TEXT FIG. 1. Apples inoculated with *Glomerella cingulata*, showing acervuli.

This fungus in nature causes bitter rot of apples (text fig. 1) always with the production of an abundant enzym-secreting mycelium and very numerous conidia-bearing acervuli and usually acervuli only, though sometimes upon apples is found a strain that bears numerous perithecia imbedded in the flesh of the apple. In artificial culture in my experience most strains isolated remain non-perithecial, but when perithecial are so only when the cultures are quite old.

Clinton (1902) who first found the perithecial stage says: "in practically all of the cultures that were made . . . there developed in time . . . the permanent stage. . . . This generally appeared . . . within two weeks after the cultures were started."

Stoneman (1898) who studied this fungus in 1898, searching particularly for perithecia, did not find any.

Clinton describes the perithecia as borne in a "stromatic cushion $1/16-1/4$ of an inch in diameter." These masses did not develop simultaneously, but in long succession. Clinton describes the perithecia as in a stromatic cushion, more or less compounded, subspherical. The conidial stage is referred to as a *Gloeosporium* and setae are not mentioned.

Von Shrenk and Spaulding (1903) say that "the perithecia are imbedded in hard masses of mycelium . . . in size from a small pin head to one-fourth of an inch in diameter . . . from one to many are imbedded, . . . there is no beak." Their figures emphasize the idea that the perithecia are borne in stromata (von Shrenk and Spaulding, Pl. V, figs. 5, 7).

It is particularly to be noted that both von Schrenk and Spaulding and Clinton describe the perithecia as in stromata and mention no setae in the acervuli.

In my own observation I find perithecia produced in the following ways:

1. In *dense clusters* of from 50 to perhaps several thousand perithecia constituting a black hard mass often reaching 5 mm. in diameter. These masses on teasing or crushing apart prove to be composed of many distinct though touching perithecia, *i.e.*, there is no stroma though these structures seem to be the same as described and figured by Clinton and von Schrenk and Spaulding as stromatic. The individual perithecia may be globose, $45-60\ \mu$ in diameter or much larger, $185\ \mu$, and then flask-shaped and beaked, making a total height from base to beak tip of $240\ \mu$.

This is the perithecial form most rarely met and occurred on only three strains and then usually only on very old cultures and usually only a dozen or so groups per petri dish or bottle culture (6 oz. slant agar). Two strains of this type of response, secured from Dr. Anderson, when placed in bottle cultures eventually made the clusters of perithecia. On irradiation, however, they produced the scattered solitary type of perithecium only and this type sometimes occurred without irradiation on the same plates that bore the dense clusters. Sometimes there are small groups, 1 mm. wide, of large discrete flask-shaped perithecia and the inter-perithecial space bears numerous very young perithecia. Twenty-six days after irradiation with the usual response of scattered perithecia some of the plates showed dense perithecial masses on the non-irradiated area and on the new growth on the irradiated side but none on the irradiated area; evidently the profuse production of perithecia due to the irradiation precluded formation of perithecial masses in the irradiated area by exhaustion. These dense groups were never produced as the result of irradiation.

2. Globose, non-rostrate perithecia produced in *small clumps*. This form was noted in only one strain and only as the result of irradiation (Stevens, 1928b, fig. 6).

3. *Scattered*, for the most part solitary, though very rarely a clump of from two to a dozen may be seen, usually very numerous, globose, non-rostrate perithecia (Stevens, 1928*b*, fig. 5, p. 220). These were the type usually produced by irradiation. Strain G 10-15 gave uniformly about 15 perithecia per square millimeter or about 100,000 to an ordinary petri dish. They also occurred regularly in certain strains without irradiation.

In certain instances a phenomenon so rare as to be almost negligible, yet so interesting as to be noteworthy, is seen. It is the occurrence of a crop of perithecia on the non-irradiated half of the plate and long after the first crop induced by direct irradiation has matured, or on very old plates that have not been irradiated. The perithecia are isolated, *i.e.*, not grouped and are very numerous though less so than those induced by irradiation, about 4 per square millimeter. In fact they precisely resemble the induced crop except as to numbers. Such perithecial crops are not common; they have been noted perhaps only six to ten times on some 500 plates. In all cases they occur among mycelium that was not irradiated, in the shaded half of the plate or in mycelium that has developed since irradiation or on plates not irradiated. They were not evenly distributed over the plate, but were more common toward the edges, as though perhaps caused by drying of the medium.

The asci and spores found in the perithecia of each of the above types were alike and agreed well in measurements with the descriptions published by others heretofore.

The dense cluster mode on two strains developed with reasonable certainty and regularity. On one other strain they occurred extremely rarely with no obvious relation to environment. All three responded on irradiation with numerous scattered perithecia. The mode of scattered perithecia was the response to irradiation in all other cases except that of one culture which gave small clumps. Scattered perithecia were produced also by several strains either with or without irradiation.

To ascertain whether greater dosage might induce perithecia a non-perithecial strain was irradiated for 1, 2, and 3 minutes. No perithecia were produced. Late irradiation on a 13-day-old colony gave precisely the same response as on younger colonies. Repeated irradiation from 1 to 5 times, 30 seconds each time, at two-hour intervals showed no difference from that of a single irradiation. No difference is noted in perithecium production due to inverting the plates. Numerous tests of mycelial and conidial inoculations were made, but no differences were noted in their behavior.

Acervuli usually develop abundantly on most strains in natural or artificial conditions though some strains are almost devoid of acervuli while in others they are very profuse. Usually the acervuli are non-setose, but in some strains setae are abundant and in some strains they appear in abundance at some times though absent at other times. Miss

Stoneman (1898) has noted the sudden appearance of setae in cultures of this fungus. Though the presence or absence of setae is the basis of separation between the form genera *Gloeosporium* and *Colletotrichum*, setae are in many species quite inconstant. Shear and Wood (1913) say that "some acervuli from one single spore culture may show many setae, others few, and still others none."

Krüger (1913) has also shown the variability in these structures and cites numerous others who have noted the same thing and says that setae in certain species may be developed at will by altering conditions.

The formation of acervuli is strongly influenced by irradiation since in many instances a distinct band of acervuli is seen in the locus of the irradiated mycelial tips (text fig. 4). In an occasional plate, acervuli are produced at the locus of the mycelial tips whether irradiated or not though in most cases this is not so. It thus appears that here irradiation hastens a natural process. Again the non-irradiated portion of a plate may be densely acervulous and the irradiated part non-acervulous. However, no law as to their relation to irradiation was observable, since there is no such regularity in the induction of acervuli as of perithecia. With varying wattage and time, it in general appears that slightly greater energy is required to induce this zone of acervuli than to induce perithecia.

Thus at 125 watts we have:

| Seconds | Perithecia | Acervuli |
|---------|------------|----------|
| 4..... | + | 0 |
| 5..... | + | Few |
| 6..... | + | 0 |
| 7..... | + | + |
| 8..... | + | + |
| 9..... | + | + |
| 10..... | + | + |

There is, however, an irregularity in the dosage required to induce acervuli that is not met as regards perithecia; for example, at 200 watts we have:

| Seconds | Perithecia | Acervuli |
|---------|------------|----------|
| 1..... | + | Few |
| 2..... | + | 0 |
| 3..... | + | 0 |
| 4..... | + | + |
| 5..... | + | 0 |
| 6..... | + | + |
| 7..... | + | Few |
| 8..... | + | + |
| 9..... | + | + |
| 10..... | + | 0 |

It is evident from numerous observations that the dosage needed to produce a marked colony stunting is less than that needed to cause zones of acervuli.

Regarding appressoria it may be mentioned that from a study of over 300 cultures one of my students, whose results are as yet unpublished, states: "My conclusion from these data is that *Glomerella cingulata* (Stonem.) S. & v. S., strains G₂ 12 and G₂ 13, produce fewer appressoria as a result of irradiation than the corresponding half of the colony which was not irradiated."

Glomerella cingulata as studied was isolated in monosporous culture from conidia from apples from various states and several pure cultures were kindly sent to me from other laboratories. In all nearly 50 monosporous cultures have been studied.

These strains in many cases varied largely from each other in culture characters, color, rate of growth, abundance of acervuli, etc., as well as in response to ultra-violet irradiation. Some produced perithecia readily on apples, others only acervuli. Some produced perithecia in equal abundance on corn meal agar plates whether irradiated or not. Some produced no perithecia in artificial culture whether irradiated or not, nor did they bear perithecia on any medium or under any dosage of irradiation tried. Still other races were never seen to bear perithecia when not irradiated though they did so profusely when irradiated. Many strains of each of these three categories have been under study and are shown in the main to maintain their character unchanged.

A culture of *G. cingulata* received from Dr. Giddings in one instance was seen on irradiation to produce dark mycelial plexi similar to those produced by *C. lagenarium*, and similarly to bear perithecia soon near these plexi, though in some instances it bore perithecial groups not associated with plexi.

From the above given statements it is seen that *G. cingulata* from apple as studied by me consists of numerous strains of very distinct differences. It is also obvious that the strains studied by Miss Stoneman, Clinton, and von Schrenk and Spaulding differed from mine. The last authors indeed mention one strain with cream colored conidia instead of the usual type (von Schrenk and Spaulding, p. 21). Edgerton (1908), Lewis (1909), and Schneider-Orelli (1912) also maintain the existence of strains.

G. cingulata from apple, as is apparent from my own studies, when grown on various media and in various environments and especially under the influence of ultra-violet irradiation is separable into over 40 distinguishable strains and it is apparent that this number could be extended almost limitlessly by sufficient search. These strains should be regarded as "elementary species" or perhaps as the jordanons of Lotsy (1916). How they may differ in climatic reaction and in virulence and in host relation is unknown.

G. cingulata as at present regarded by the taxonomist and pathologist, accepting the conclusions of Shear and Wood (1913), occurs on a very large

range of hosts. It is interesting to speculate as to the number of jordanons that might be isolated from each of these hosts and as to whether any jordanon, for example from apple, is identical with any of those found on the other hosts.

MUTATIONS

In April, 1928, the author sent to press a paper (1928) recording the reactions so far as then known of 13 strains of *Glomerella cingulata* to ultra-violet irradiation. During the summer these strains were carried on slant corn meal agar in 6 oz. bottles and transfers were made about once a month. On my return to work in late September, 1928, it was found that very material changes had occurred in certain of these strains in their responses to irradiation. Strain G 10 on which most of the results reported heretofore were obtained and which in all earlier work had made no perithecia, or very rarely any, without irradiation, and had always responded with very numerous perithecia when irradiated, was now altered. While several of the cultures still responded to irradiation precisely as they did in June, some transfers of this strain did not, in that they produced perithecia on both the irradiated and the non-irradiated portions of the plate, or failed to produce them at all. This occurred consistently on several consecutive transfers. The changes in character noted above had occurred at some unknown time during the summer.

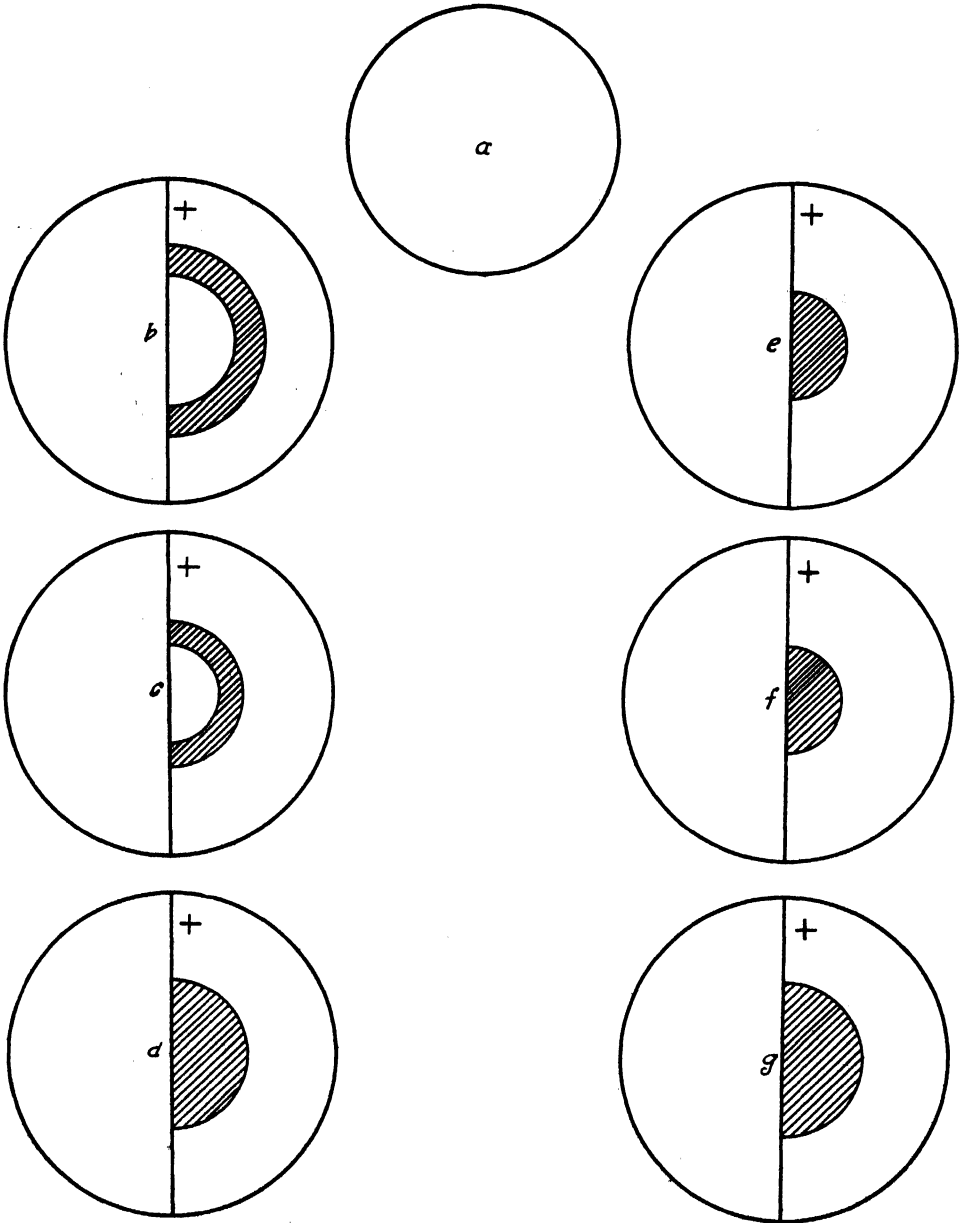
A transfer of G 10 made 10-22-'28 to a petri dish was seen to make perithecia in both the irradiated and the non-irradiated regions, but only in well-defined sectors that remind one of these noted in *Helminthosporium* and many other fungi (Stevens, 1922). Transfers made 11-1-'28 from the non-irradiated perithecial sector, produced perithecia both in the irradiated and non-irradiated regions. Transfers from the non-perithecial region gave colonies that remained non-perithecial even when irradiated.

Thus G 10 consisted of three strains: (1) that of the original character, *i.e.*, perithecia present only on irradiation; (2) colony non-perithecial whether irradiated or not; (3) colony perithecial whether irradiated or not.

In recognition of this evidence of variability pedigree cultures were then undertaken and the following are the most significant results:

G 10-3 which gave a fine perithecial response on irradiation was seen to consist of sectors. In one of these, G 10-8, the perithecial response was confined to the older mycelium within a radial distance of about one centimeter from the center of the colony. The sector G 10-9 differed in that no perithecia were produced in this inner region, but they were produced in a zone about 1 cm. wide, the outer edge of which corresponded with the position of the mycelial tips when irradiated (see text fig. 2). In other words, G 10-8 was responsive in the mycelium that was three days old when irradiated; G 10-9 in the mycelium that was about one day old when irradiated. Precisely this character was maintained in the next transfer, but the character was lost in the next transfer.

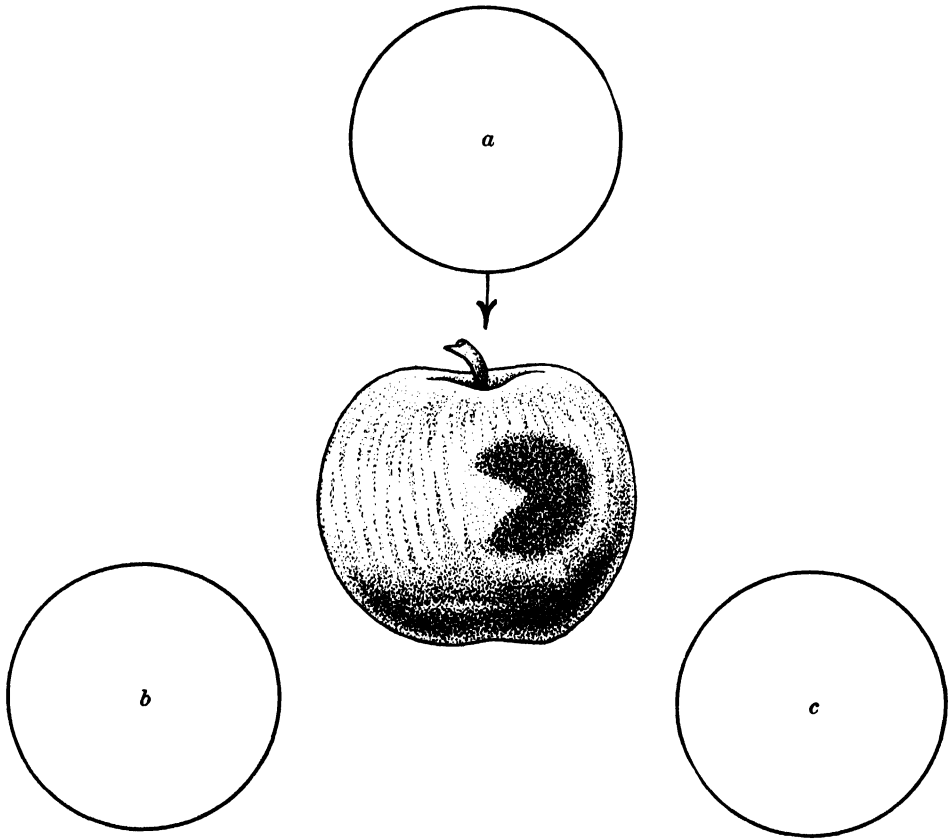
In two other instances, G 10-10, G 10-11, this same sectoring was observed and in both cases the character was maintained through only a few transfers and was then lost.



TEXT FIG. 2. Diagrammatic representation of the changes noted in G 10-3. The shaded regions gave perithecia on irradiation. *a*, G 10-3; *b*, G 10-9, which sectoried from G 10-3; *c* and *d*, later cultures of G 10-9 showing change in perithecial region; *e*, G 10-8 which sectoried from G 10-3; *f* and *g*, later cultures of G 10-8.

G 10-b arose as a sector from G 10, 1-5-'29, as a non-perithecial strain. It remained so for several transfers, when it began to produce a very few perithecia on the irradiated side (12-17-'29).

G 10-16 arose as a non-perithecial sector (11-12-'28) from G 10-5 which gave perithecia either with or without irradiation. This remained true to its new character when last observed, 11-16-'28.

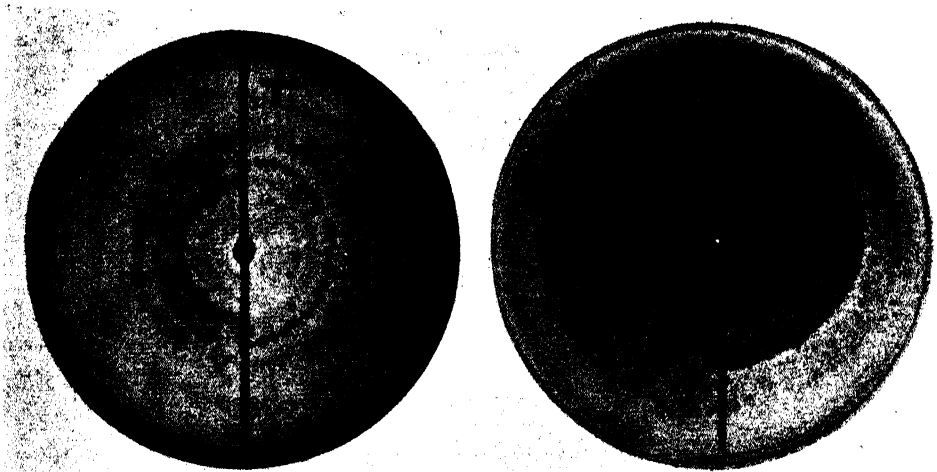


TEXT FIG. 3. *a*, G 7 which was perithecial on irradiation and which sectoried on an apple giving *b* which is G 7-1, pale, acervulous and perithecial on irradiation, and *c* which is G 7-2 dark, non-acervulous and with perithecia with or without irradiation.

G 7 which gave perithecia whether irradiated or not was inoculated upon an apple and it there sectoried giving two strains of very different characters; G 7-1 was a very pale, tan colored sector bearing numerous pink acervuli; G 7-2 was black and bore no acervuli (text fig. 3). On irradiation G 7-1 produced perithecia only where irradiated, while G 7-2 produced perithecia whether irradiated or not, though there was an increase in number of perithecia in the younger irradiated zone (text fig. 5). These two strains retained their characters perfectly through many transfers and were true when last tested, 3-19-'29. This case is of especial interest as an instance of mutation in the natural habitat of the species.

G 2-14 originated, 11-15-'28, as a pale sector bearing very few perithecia. It remained true through many transfers and was unchanged when last tested, 3-19-'29.

G 2-12 sectoried out, 10-28-'28, as a non-perithecial strain and remained true through many transfers and was still true when last tested, 3-19-'29. G 2 appears to be a very variable strain in that many variant sectors appear upon plates of it. G 2 in June was like G 10 in irradiation response, but its transfer in October gave numerous perithecia either with or without irradiation. In a plate inoculated 11-5-'28 two sectors differed markedly (see text fig. 6). One of these, G 2-4, a dark strain, gave no

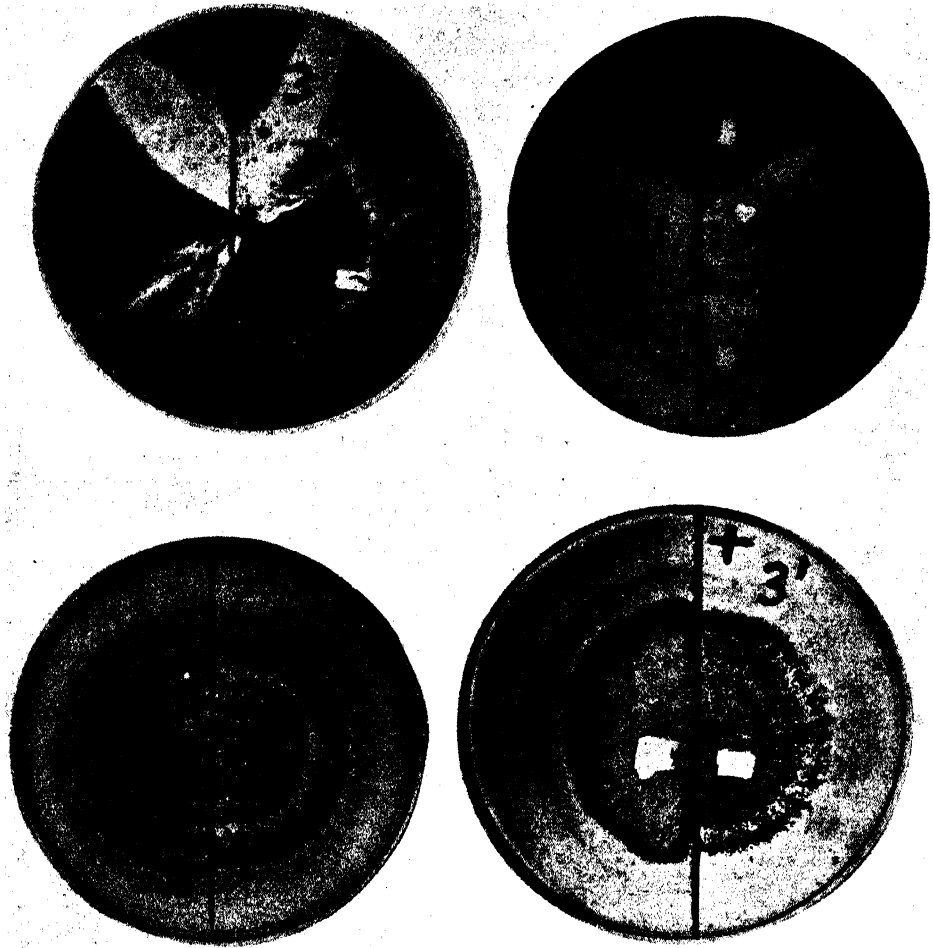


Text FIG. 4 (left). G 10-15, 2 amp., 2", showing acervuli on the irradiated mycelial tips. FIG. 5 (right). G 7-2. A sharp line is formed by perithecia on the irradiated side, but perithecia continue to form on the new growth beyond this line as they do in the non-irradiated side. The congestion of perithecia in the young growth that was irradiated shows some response here by this strain.

perithecia at all; the other, a pale strain, G 2-3, gave perithecia only when irradiated. G 2-3 remained true through four transfers, but the other in four transfers reverted to its former condition.

G 2-13 appeared as a non-perithecial sector, but in the first transfer it produced a few perithecia whether irradiated or not. This continued through six transfers, but when tested, 2-13-'29, it was non-perithecial. G 2-b was perithecial even when not irradiated. It sectoried into G 2-7 which was quite non-perithecial and G 2-6 which produced perithecia. G 2-3 arose as a hyaline sector from G 2 which was perithecial with or without irradiation. The sector G 2-3 was perithecial when irradiated, but not otherwise. It after several transfers reverted to the type of G 2. Several other sectors from G 2 were from time to time tested, but none of them remained permanent (text fig. 7).

G 9 on Nov. 8, 1928 gave two sectors one of which, G 9-1, bore no perithecia even when irradiated; the other, G 9-2, a dark colony, produced abundant perithecia whether irradiated or not, but with an increase in number of perithecia in the region of the young mycelium (text fig. 8).



TEXT FIG. 6 (left). G 2, showing origins of G 2-3 and G 2-4. FIG. 7 (right). G 2, showing several sectors. FIG. 8 (left). G 9-2, showing increase of perithecia on irradiation. FIG. 9 (right). G 10-13, irradiated, showing perithecia on the irradiated side much larger than on the non-irradiated side.

G 9-2 maintained its character through many transfers until 1-27-'30 when last tested, but G 9-1 on the second transfer bore a very few perithecia on the irradiated part of the colony, but none in the non-irradiated part. The next transfer gave more perithecia, and the next gave a full perithecia response on the irradiated portion and none on the non-irradiated portion. This behavior of G 9-1 seems to give an example of a strain that gradually changed in its capacity of sexual response to ultra-violet irradiation.

G 10-13 arose as a sector of G 10 as a strain that produced perithecia either with or without irradiation. It maintained this character through many transfers and when last tested, 2-25-'29. When this strain was irradiated with dosages varying from 2 seconds to 6 minutes, the short exposures of two seconds and 30 seconds give no noticeable results, but with one minute irradiation the colony was darker 24 hours after irradiation (text fig. 9) due apparently solely to increase in size of the perithecia though there was no change in their number. While the perithecia on the non-irradiated area averaged $30\ \mu$ in diameter those on the irradiated side averaged $47\ \mu$ and were covered with a fine profuse radiating mycelium. With three minutes irradiation the perithecia were even larger, $77\ \mu$, though no asci were found in them. It appears probably that irradiation with large dosage on perithecia already developing causes them to revert to the vegetative stage.

Quite frequently it was observed that in certain cultures the perithecia, while in general of the scattered type, in certain spots on the plates bore perithecia in dense botryose clumps. In other instances one main hyphal thread was especially peritheciigerous resulting in rows of perithecia 1 to 2 cm. long. This grouping of perithecia whether lineally or in clumps is a variable character that often persists through only a few transfers, G 7, but eventually becomes lost.

Five single conidiospore isolations were made from agar plates of G 10-5 in November, 1928. All of these responded precisely as did G 10-15 to irradiation, with many perithecia on the irradiated part of the colony and none on the non-irradiated part. Thus, there was no evidence of mutation.

Three single ascospores were isolated from perithecia of G 10-15 that were produced as a response to irradiation and were cultured as G 51, G 52, and G 53. Each of these kept under observation for months gave the usual response to irradiation and could be distinguished in no way from cultures of G 10-15, thus showing no evidence of genetic change due to the irradiation.

From all of these observations upon *Glomerella* strains it is clear that the strains by sectoring give rise to new strains which may differ in color of colony, pale to dark, abundance of appressoria, abundance of acervuli, and in perithecial response to irradiation. Some of these variants may remain true to their new characters, others may gradually or suddenly change back to their original character.

SUMMARY

Perithecia are produced in three ways: dense clusters, small clumps, scattered solitary.

Setae are sometimes abundant.

Acervuli formation is strongly influenced by irradiation. About 50 monosporous cultures were studied. Some produced perithecia readily, others only acervuli. Some bore perithecia whether irradiated or not.

Some produced no perithecia, others bore perithecia only when irradiated. Thus numerous strains are recognized.

Mutations by sectoring occur resulting in changes of character, especially in response to irradiation. One such mutation was observed to occur on apple.

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STUDIES ON MORPHOGENESIS IN *AGARICUS* (*PSALLIOTA*)¹ *CAMPESTRIS*²

ILLO HEIN

(Received for publication April 25, 1930)

The mycelial vegetative stage of *Agaricus campestris*, which precedes the production of basidiocarps, I have described in a previous publication (1930, b). Morphological accounts of the fruiting stages are more abundant in the literature of the fleshy fungi than for any other type unless it be of *Coprinus*, but while the data are voluminous, they are still far from complete and there is much still to be described concerning the development of these forms. The histogenetic and morphogenetic processes involved in the aggregation of the cylindrical, tapering hyphae and the determination of their ultimate interrelations in the pseudoparenchyma, plectenchyma, prosoplectenchyma, or other mature tissue in the ripe fruit, and the factors which accelerate or retard their growth, branching, interweaving, septation, orientation, expansion, contraction, and fusion, have been practically untouched. There still is much to be said about their development into gills and pileus.

Few data are available concerning the transition stages from mycelium to carpophore: whether this is initiated in a substitute sexual process or is an environmental response is not yet known. The questions concerning heterothallism have been little studied in *Agaricus campestris* and while data have been recently given to show that the two-spored commercial varieties are homothallic (Lambert, 1929), no such studies have as yet been reported for the wild, four-spored forms.

LITERATURE

Carpogenesis. The environmental conditions which are associated with the initiation of the transition from the vegetative to the reproductive stage in the fleshy fungi have been discussed by Brefeld (1877), Klebs (1900), Falck (1902), and others and the main facts have been reviewed by Magnus (1906). Magnus states that exhaustion of food materials from the substrate and the transition to an aërial environment probably are the main factors concerned. He has suggested that accumulation of acids and excess of carbon dioxide and degree of vapor pressure may also play a part in initiating

¹ In conformity with the nomenclature in the recently published *Icones Farlowianae* (1929), which may be regarded as standard, the writer feels that it is desirable to return to the use of the older name *Agaricus*.

² Publication authorized by the director of the Pennsylvania Agricultural Experiment Station as technical paper no. 491.

fruit production. Magnus observed that in poorly ventilated cellars the mycelium will run for days over the bed surfaces without the production of carpophore anlagen and that, therefore, change to aërial environment alone cannot be the sole factor. Over areas of beds in which the mycelium was "running" well, he placed bell jars but only a loose felt developed under them while outside the jars numerous anlagen appeared, indicating further that lack of air does not induce fruiting.

The environmental factors concerned with fruit production have been more intensively studied in the lower microscopic fungi and here it has been claimed that the concentration of food stuffs in the medium (Milburn, 1904; Klebs, 1900; Riedemeister, 1908), darkness (Gallermaerts, 1910; Munk, 1912), hydrogen-ion concentration (Milburn, 1904), osmotic pressure (Riedemeister, 1908), and certain chemicals (Molliard, 1910; Heald and Poole, 1909) are the factors associated with the initiation of the reproductive stages.

Development of the Basidiocarp. The foundations for the study of the growth, development, and morphology of the basidiocarp were laid by Hoffman (1860 and 1861), de Bary (1884), Hartig (1874), Brefeld (1877), and Fayod (1889) and their work as well as that of later investigators has been adequately reviewed by Magnus (1906), Atkinson (1906 and 1916), and Levine (1922). More recently the literature has been summarized by Gäumann (1926). On the basis of his own work and that of his predecessors, de Bary presented the first connected account of the development of *Agaricus melleus* from the time the young fruit body first appears to the stage when the mature tissues are fully developed.

The most adequate study of the form and structure of the basidiocarp of *Agaricus campestris* was made by Magnus (1906). According to Magnus the young anlage is an elongated ovoid to spheroidal body consisting of irregularly intertwined hyphae, assumed to be negatively geotropic and generally mounted upon a thick rhizomorphic strand. The negative geotropism of the hyphae Magnus states may play a part in the early differentiation of the basidiocarp.

OBSERVATIONS

Very little of the fully developed tissues in the basidiocarp of *Agaricus campestris* becomes even in the mature stages a true pseudoparenchyma in the sense that the cells appear isodiametric when sectioned in three planes. The individual long, cylindrical hyphal cells are present throughout all stages.

The tissues of the fleshy fungi are commonly referred to as pseudoparenchymatous and while here and there this kind of tissue may be found it is by no means common in the forms which I have examined. No doubt the mycologists who have so described the tissues of fleshy fungi are fully aware of the actual form of the hyphal cells but have lacked uniformity

in their use of terminology. The need for clarity and establishment of limits in the use of terms is obvious and since many of them have been plainly defined the need for repetition of definitions here would appear superfluous. Since, however, there is a very frequent loose usage and at times misuse of some often employed descriptive terms I shall attempt to state clearly the meaning of such mycological expressions as I understand them from the literature and shall use them in the present writing.

A *pseudoparenchyma* is a parenchyma-like tissue, a "Scheingewebe" (de Bary) and differs from a true parenchyma only in its method of development (symphyogenetic) and the plant groups in which it is found. A parenchyma as generally defined in the literature (Sachs, p. 83, *et al.*) is a tissue which consists of thin-walled, protoplasmic, polygonal, isodiametric cells. A pseudoparenchyma is similar to a parenchyma but is formed by growth, interweaving, branching and septation in only one plane (symphyogenetic) of tubular cells. Gäumann (1926) in defining various fungous tissues states, ". . . haben die einzelnen Hyphen und Zellen ihre Individualität verloren, so dass sie auf Querschnitten gleich den Zellen der höheren Pflanzen isodiametrisch und lückenlos nebeneinander liegen so spricht man von einem Pseudoparenchyma," and illustrates such a tissue with a reproduction of Von Tavel's figure 2a (1892). In most fleshy fungi the hyphal cells retain more or less their cylindrical shapes and in accordance with the above definition cannot be the elements of a pseudoparenchyma. I shall use pseudoparenchyma only for the symphyogenetic tissues whose cells are essentially isodiametric.

A *prosopectenchyma* as defined by Gäumann is a plectenchyma in which the individual elongated hyphal cells are still recognizable. De Bary's figure 14 (1894) and Von Tavel's figure 3b (1892, p. 83) represent such a tissue and this is the kind most commonly found in the fleshy fungi. Lindau (1899), proposed *prosopectenchyma* for prosenchyma-like hyphal tissue. The term is a useful one and should be employed especially for such tissues as occur, for example, in the pileus of *Agaricus campestris* where the cells are in general oriented in various directions and cylindrical in shape. Plectenchyma (Lindau, 1899) is commonly used as a more general term and includes all tissues which have originated from filamentous (hyphal) cells.

For the kind of plectenchyma found in the stipe and in the strands of basidiomycetes where the cells are elongated but more or less parallel, no satisfactory expression has as yet been suggested. Orton (1924) has proposed *pseudo-palisade parenchyma* but palisade implies that the parallel cells are arranged so as to appear in rows when sectioned. For such tissues Orton's term is applicable. In Starbäck's diagram various types of plectenchyma, based on the form and orientation of the hyphal cells, are represented and named. The plectenchyma made up of elongated parallel hyphal cells is described by Starbäck as "langgestrecktes Plectenchyma."

Other kinds of plectenchyma represented in Starbäck's diagram are: *prismatic* plectenchyma, which consists of a densely compact tissue in which the cells are oblong in section and while they are not isodiametric may be classed under pseudoparenchyma; and *spherical* plectenchyma, where the cells are more or less isodiametric but loosely aggregated and spherical in shape. Such spherical cells are common in the gills of *Agaricus campestris* at the base of the basidia. *Verflochtenes* plectenchyma consists of profusely branched, loosely interwoven hyphae and is typical of Lindau's proso-plectenchyma. *Verklebtes* plectenchyma is less branched, the hyphae are not as irregularly oriented but show a slight tendency to be parallel with apparent adhesion. The diagram does not make entirely clear just what is meant by this kind of tissue. The *langgestrecktes* plectenchyma in Starbäck's diagram I have mentioned above. Finally there is the *epidermoides* plectenchyma in which the hyphal cells are closely packed, somewhat isodiametric, but slightly gnarled and apparently thicker walled than any of the above. Whether this last type corresponds to a paraplectenchyma was not made clear.

Since Falck (1909), followed by Bensaude (1918), Kniep (1915, 1917, 1919), and others distinguished primary and secondary mycelium on the basis of cytological characteristics without apparently considering the earlier use of the expressions by de Bary, Woronin, Harper, and others, the terms have come into general use. According to present usage by the students of homothallism and heterothallism, primary mycelium is the multinucleated or uninucleated haploid mycelium and is followed by the binucleated, diploid, usually clamp-bearing condition, which is called secondary mycelium. Tertiary mycelium is defined as the hyphal tissue which originated from the secondary mycelium and includes all hyphal systems which have become specialized as rhizoids, carpophores, strands, sclerotia, etc. The earlier workers used secondary mycelium to describe the mycelium which is produced by fruiting structures and is nutritive in function. Fayod (1889) made an elaborate classification of fungous mycelia and states that primary mycelium is the filamentous mycelium during the stages from spore germination to the production of old mycelium, and secondary mycelium is that which is produced from the base of carpophores, ascocarps, etc. Gwynne-Vaughan (1930) suggests *monothallic*, for the mycelia of a single strain which arises from one spore and *dithallic* for the mycelia arising from the union of + and - strains. These terms correspond, respectively, to the recent usage of primary and secondary mycelia and avoid the conflict with the earlier significance of the latter expression. I shall in the present writing use *secondary mycelium* in the older prior sense and try to avoid the modern significance of the term.

A tissue which consists of actively dividing cells is generally spoken of as being meristematic and in this broad sense would include all growing regions. A more restricted use of this word has been made in the fungi.

In developmental studies of pycnidia by Bauke (1876), de Bary (1884), Von Tavel (1886), and Schnegg (1915) the development is spoken of as "meristogen" when the cells grow and divide in all directions and "symphyogen" when filamentous cells grow, branch, interweave, and divide in only one plane. This is in my opinion a useful restriction of the terms and I shall henceforth use them only in the above sense. In the authorized translation of de Bary, "symphyogen" is translated as "symphyogenetic" but a corruption of the German translation to "symphogenous" has appeared in the literature.

At this point it may not be out of place to point out that the cases in which meristogenetic development is supposed to have taken place in the development of the pycnidium (Bauke, de Bary, *et al.*) and in the apex of the rhizomorph (Hartig, 1874, as discussed by de Bary, 1884) are actually cases of interweaving and branching of very short hyphae. That in the studies quoted, the development is meristogenetic is not at all convincing and I am of the opinion that there is no case of actual meristogenetic development in the above sense in any fungus tissue yet described. Division in one plane is a well-known characteristic of fungous cells and where division has been described as occurring in more than one plane the cases are in reality the production of short branches which through adhesion and interweaving present an appearance of being meristogenetic.

Morphogenetically the stages in the development of *Agaricus campestris*, up to the basidiocarp initials, may be classified under the following heads and I shall discuss them in this order: the spore, germination, the hyphae, the mycelium, strands (or rhizomorphs), centric growth, carpogenesis, and the bi-polar pin-head.

The Spore

While the spore has been many times described as a symmetrical ellipsoid, more critical examination shows it to be somewhat asymmetric with a slight tendency to be kidney-shaped. Hence the outline of the spore will vary depending on the plane from which it is viewed. Looked at from a direction along a line which connects the tips of the two sterigmata, the outline of the spore is a fairly perfect oval with a slight tendency to be broader at the base. Viewed from a direction at right angles to this the spore as it is poised on the sterigma is slightly kidney-shaped. In transverse section the spore is approximately circular in outline. Most text book figures and figures in the literature picture the spore as perfectly ellipsoidal in form. It is beautifully symmetrical but by no means exactly of this shape. The spore appears to be balanced on the sterigma so that the axis of support passes through its center of gravity. The form and development of the spore will be described in full at a later date.

Spore Germination

A summary of the literature and a description of spore germination in *Agaricus campestris* have already been given (Hein, 1930b). Germination begins with the production of a colorless, spherical vesicle which may arise from any part of the cell. From one to three germ papillae are produced and they enlarge until a diameter equal to the smaller dimensions of the spore is reached when they proceed to elongate into typical cylindrical hyphae. This germ tube is frequently somewhat irregular in its cylindrical form and from it the hyphal branches grow in various directions.

The Hyphae

The hyphae are gnarled but fairly cylindrical from the beginning. The general direction of growth is, as in other fungi studied, radial from the spore as a center (Hein, 1928 and 1930b). While the general direction of all the hyphae is more or less radial, secondary and later branches and occasionally primary branches may be oriented here and there in all possible directions. Cell fusions between the hyphae are common in all stages of development and may occur as end-to-end or as lateral anastomoses between hyphae produced from the same or from different spores. The radial growth directions of the primary hyphae may be determined by some such factor as morphaesthesia as has been previously suggested (Hein, 1928) but the orientation of secondary and later branches may be determined by nutritional stimuli. This is shown by the manner in which the hyphae crowd into unoccupied space.

The gnarled shapes of the hyphae are the result of slight constantly varying growth directions. That the slight variations from the main path are not caused by mechanical barriers is indicated by the fact that even in a fairly homogeneous medium such as distilled water the gnarled shapes occur. Possibly minute variations in nutritional stimuli, repeatedly varying responsiveness in the hyphae, or rectipetioses cause the slight excentricity in the growth directions which give the gnarled shapes.

The Mycelium

Mycelial development is progressively centrifugal away from the spore or from the center of inoculation. At this stage the growth directions of the organism are presumably controlled largely by nutritional stimuli which continue until a certain period of development is attained (when the substrate is more or less completely interpenetrated) and the feeding and growth stage is at a climax. With the termination of this vegetative stage the mycelium has arrived at what is crudely called, maturity. Carpo-genesis is initiated and a reversal in centricity occurs involving a change in what may be called "Stimmung" (Harder, 1929). The change in "Stimmung" is to be regarded as an adaptation which, according to Parr (1926), is an adaptive relationship involving both the organism and its

environment. The organism, the mycelium in this case, no doubt causes certain changes to take place, an alteration in its physical and chemical composition, in the substrate during the feeding and growth stage. During this stage chemical substances are taken in by the mycelium until a degree of exhaustion is reached. Presumably, so long as a certain optimum of nutritive substances is present in the substrate, growth will be toward that optimum but below this point a change occurs in the relationship between the mycelium and its environment (substrate). Change in "Stimmung" may, of course, be entirely independent of external environmental conditions and may be due to internal, rektipetiotic (Pfeiffer, 1925) factors expressed in what we call maturity and is apparently exemplified in higher animals. The stimuli are then to be thought of as different and will presumably call forth correspondingly different responses. Whether reproductive maturity is to be regarded as due to nutritional adaptiogenesis, that is, a succession of morphotic and functional adaptations in response to changing environmental stimuli as appears to be the case in many protozoans, or is an inherited cyclic characteristic, remains to be determined. Klebs (1904) has given data which tend to show that the concentration of foodstuffs especially carbohydrates is of importance in bringing about fruiting in fungi.

The cyclic succession of basidiocarps in mushroom beds in commercial mushroom houses is a familiar fact to the growers and may be the manifestation of inherited internal periodicity. Possibly this cyclic fruiting is due to conditions in the substrate. For example, staling products may be produced in the beds which, when they reach a certain concentration, inhibit further fruiting. After a period of non-production these products may be dissipated and conditions then become favorable again for further fruiting. Another possibility to be considered is that the mycelium more or less completely occupies the substrate and remains in the fruiting state until it is terminated by senescence, degeneration, and decay. When the degeneration has reached a degree of completeness there is again space and favorable environment for renewed growth. The new mycelium may arise from remaining viable mycelium which one may look upon as centers of inoculation, or even from spores shed from the previously produced basidiocarps. The alternate bearing and non-bearing periods may thus be regarded as a succession of life cycles. Still another possibility to be considered is that during the bearing period available nutritive substances are rapidly and fairly completely used up and fruiting ceases through starvation. During the non-productive period chemical and physical changes occur in the substrate resulting in the production of available nutritive substances and fruiting recommences.

In a new mushroom bed the fruit bodies first appear in clusters at the centers of inoculation and from these centers an ever widening more or less circular patch is produced. The basidiocarps thus spread radially

from the patches as the mycelium becomes progressively mature. Finally the whole bed becomes covered with a fairly uniform stand and the centers of inoculation are no longer conspicuous. When the second and later "breaks" occur the fruit bodies come up more uniformly as regards their distribution. Clusters do arise here and there but they are not limited to the centers of inoculation.

I have never observed that the fruit bodies occur in the mushroom beds in periodic rings or even in a single ring as they do in the field under natural conditions. Periodic environmental conditions are no doubt eliminated in mushroom houses because of the artificial methods of culture. Under natural conditions there is an annually widening ring (Shantz and Piemeisel, 1917) and the locality may remain undisturbed for years while under artificial cultivation beds are made up and taken down annually, the beds remaining in the houses considerably less than a year. Since the concentration of available nutritive materials has been shown to be a factor in ring production (see literature cited in Hein, 1930a) this factor probably could not in so short a period come to expression in the richly nourished artificial beds. It would be interesting to keep mushroom beds under favorable environmental conditions for a number of years and compare them with mushroom-producing areas in the fields.

The Rhizomorphs

The rhizomorphs actually begin to develop when the first fusions occur between the hyphae. This commonly happens shortly after the hyphae emerge from the germ tubes (Hein, 1930b). Growth with branching at acute angles, interweaving, adhesion resulting in parallel growth, and fusion of the hyphal cells cause the formation of the strand tissue. While the strand may be regarded as an adaptive response to provide conductive channels for the transport of nutritive materials to the developing basidiocarp, its formation begins so far in advance of carpogenesis that it can hardly be associated with maturity or change in "Stimmung" or other possible factors correlated with fruit production, but is a gradual parallel aggregation of hyphae which form through adhesion, anastomoses, and contact and pressure, the typical strand tissue.

Centric Growth

Before the centers of carpogenesis are initiated the mycelial growth and strand development, and presumably the movement of food materials, are in centrifugal directions. After the climax of the feeding and growth stage there is some evidence of a centripetal behavior as is expressed in the developing basidiocarp. Harper (1926) has described the centric behavior of myxamoebae which during the feeding and multiplication stages move in various unoriented directions until a period in their cycle is arrived at in which their creeping movements become centripetally directed. The

myxamoebae then creep toward the developing sorophore. "Maturity" marks the end of the multiplication cycle and is followed by the reproductive stage. The change in "Stimmung" in the mycelium of fungi is comparable to the change in "Stimmung" indicated in the slime moulds. The centripetal "flow" of nutritional substances to the developing basidiocarp is functionally analogous to the centripetal creeping movements of the myxamoebae. The centric behavior in each case is concerned with the reproductive cycle and involves the formation and nutrition of the fruiting body.

The rhizomorphs are stoutest usually near the carpophore anlagen and are progressively smaller in diameter away from them. It would appear that growth activity is therefore greater in centripetal directions. Since, however, the strand is formed by symphyogenetic growth involving profuse branching and continuous more or less equal peripheral hyphal accumulation (Hein, 1930b), the oldest, first formed parts of the strand would naturally be the stoutest. Whether a secondary mycelium is produced by the basidiocarp as in *Sordaria* (Woronin, 1869) and the mildews (Harper, 1894, 1896, 1905; Hein, 1927) has not been determined. Centripetal growth does not appear to be a characteristic of the substrate mycelium but formation of the basidiocarp involves a centripetal "flow" of nutritive substances and a centripetal aggregation, branching, and growth of hyphae. The so-called "running stage" of the mycelium, the feeding and growth cycle, ends with the initiation of carpogenesis and while mycelial development does of course continue along with fruiting, the main mycelial mass is first developed. Clumps of basidiocarps first appear above the centers of inoculation but later may occur in any part of the bed. Are these clumps produced because of possible localized areas of optimum nutritional, moisture, or other environmental conditions or are they an expression of the simultaneous change in "Stimmung" of certain hyphal groups? The clumps may on the other hand be produced only on more productive mycelium which differs inherently from that which does not produce such clusters.

It is possible too that there may be strains of mycelia in the present commercial spawns which differ cytologically. The cytological studies recently reported by Sass (1929) on species of Agaricaceae in which two-spored monothallic (homothallic) forms as well as two- and four-spored dithallic (heterothallic) forms were found in a single species suggest that similar conditions may prevail in the cultivated mushrooms. Lambert (1929) recently reported that the commercial varieties which he studied are homothallic but it is possible that there are among them dithallic forms which are productive of more numerous basidiocarps and it will be of interest to know whether the basidiocarps from clusters differ cytologically from those which do not occur in clusters. This also suggests that the numerous basidiocarps which never grow beyond the pin-head and shoe-button size may be aberrant monothallic haplonts.

Little promise of improvement through breeding experiments can be given if the present commercial mushrooms are all monothallic. The well-known hybrid vigor that results from the crossing of certain strains of higher cultivated plants suggests the desirability of breeding experiments with dithallic forms of *Agaricus campestris*. The wild species is known to be generally four-spored and by analogy with Sass's observation in *Coprinus*, etc., may be dithallic. Certainly the four-spored form should be brought under cultivation and single-spore breeding experiments made.

Carpogenesis

The earliest stages of basidiocarp anlagen are difficult to recognize with certainty in the tangled mycelial growth. The beginning stages are more or less loosely aggregated bundles of branched hyphae which are similar in appearance to the growing tips of mycelial strands. The latter are usually more loosely aggregated and can generally be recognized but this characteristic alone is too uncertain for positive determination. The hyphae themselves are similar in appearance in both strand and basidiocarp mycelium. Cell fusions have not been found in the basidiocarp tissue although they are abundant in the substrate mycelium. This characteristic has sometimes been useful as a distinguishing factor.

The first formed young basidiocarps as stated usually grow in clusters at and about the center of inoculation. From one to a dozen or more anlagen may appear within a square centimeter area and they are frequently so closely crowded that competition for space often results in irregularities in the typical form. Few of the first formed anlagen actually develop to mature basidiocarps. They perish before or at the time they reach the size of a shoe button, about half a centimeter. The earliest stages visible with a lens appear as minute, spherical aggregates of irregularly intertwined, profusely branched hyphae of diameters varying from 5 to 15 microns and they may appear either on the surface or below the surface of the substrate. The distance below the surface at which the anlagen will appear may vary from one to two centimeters. In uncased beds they are often at much greater depths. In loosely made up beds it sometimes happens that basidiocarps develop five or six inches below the surface. This observation agrees with Magnus' (1906) suggestion that the atmosphere is of importance in initiating fruit production.

For convenience the young stages may be roughly classified into three groups according to size. The earliest stages visible to the unaided eye are often called "pin-heads" by the commercial growers. In this group we may include all stages which are below two millimeters in diameter. Sizes about one half centimeter may be called the "shoe button" stage while in the well-known "button" stage those of about one centimeter diameters may be included.

Levine (1922) has already pointed out that the number of basidiocarp

anlagen produced is out of all proportion to the number of mature mushrooms which develop and states that those which are destined to die off early soon become abnormal. From the appearance of shoe-button stages, he was able to predict which ones were destined to grow to maturity and which ones would degenerate. This early degeneration may possibly be the consequence of competition for nutritive materials. When carpogenesis begins the strand mycelium may not yet have reached a stage of development for the adequate transport of nutritive materials to supply the number of anlagen produced. The chance advantage given certain favorably placed young basidiocarps may initiate a "flow" of nutritive materials in their direction which may work disadvantageously to the less favored individuals and even cause a withdrawal of such substances from them. The slightly shrunken appearance of some shoe buttons suggests this possibility although these may be after all, as suggested above, aberrant haplonts in early stages of necrosis. The development of the mycelium and the conductive efficiency of the strands may increase more rapidly at later stages or are perhaps stimulated by the demands made by the growing basidiocarps. Subsequent development of mycelium and of vascular hyphae in the strands then presumably keep pace with the rapidly developing fruiting bodies. Fruiting bodies are most commonly produced anywhere on the strand or from the termini of short strand branches and rarely at the ends of strands as in Sachs's figure 226 (1882). Tapering strands often radiate from the base of the basidiocarps and are numerous in later stages although commonly in the larger, late stages there is but a single, stout strand connecting the fruit with the substrate mycelium. The strands form, as previously described, a branched network which is anastomosed and interwoven with the mycelial complex in the substrate and basidiocarps are found developing on all parts of it separately or in dense clusters, crowding each other frequently and sometimes fusing and growing together in this fashion to maturity. The atypical fused fruiting bodies are, however, unusual.

At the time when fairly large basidiocarps are on the beds the strands are commonly very large in diameter (in some places 2 to 4 mm.) and have a more well-developed vascular system than in the earlier stages when the anlagen first appear. In the later stages the efficiency of the strands as conductive channels for nutritive substances, if this is the limiting factor in determining the number of basidiocarps, certainly is adequate for the nutrition of the numerous anlagen which are first produced if we compare their total volume with that of the bulk of a single centimeter button. It might, therefore, be supposed that many more mushrooms would grow to maturity if the numerous anlagen which first spring up were to appear after the mycelium is developed to a stage adequate in conductive capacity for their support. That this is not the case is shown by the continued appearance of excessive numbers of pin-heads in the later stages of the bed which for some reason, possibly any of those suggested above, fail to grow beyond the shoe-button stage.

Fructification in crust-like forms such as *Stereum*, *Hypochnum*, *Corticium*, and related genera, involves a widespread change in a considerable area of the surface of the vegetative mycelium. In the forms producing a basidiocarp specialization has taken place in the resupinate crust of hymenium-bearing mycelium with the formation of well organized, highly specialized structures bearing the hymenium in a protected space which is at the period of spore discharge more adequately adapted to spore dissemination. Whether this burst of fructification is initiated in a single hypha or in a hyphal region leads back to the old question of a possible carpogone. Reess (1876) described for *Coprinus stercorarius* short lateral branches of two different kinds which were presumably male and female sexual cells. A short septate hyphal branch called a *Stäbchenträger* produces numerous small both lateral and apical cylindrical to tenpin shaped cells, *Stäbchenzellen*, which may be spermatia. The latter he states were frequently found resting upon another kind of lateral hyphal cell of somewhat larger diameter, rich in cytoplasmic contents, and comparable to the "carpogone" of *Ascobolus*. The *Stäbchenzelle* when attached to the *Stäbchenträger* was well filled with cytoplasm but when found resting upon the supposed carpogone was empty as though its contents had been sucked out by the carpogone. Reess suggests the possibility that in this species there are sex cells and a fertilization process which initiates carpogenesis.

No comparable specialized hyphae have been described for *Agaricus campestris* and my own observations indicate that the basidiocarps originate as aggregates of hyphae and that no evidence of any kind of possible sexual processes have so far been found. Not even clamp connections are present in the two-spored commercial varieties and if there is a substitute sexual process associated with some of the hyphal fusions described (Hein, 1929 and 1930b), studies to date have not yet indicated this. All the cultivated varieties studied are two-spored. The wild species, said to be four-spored, have not yet been studied, and it is not known whether they do or do not bear clamp cells.

The only visible expression of the factors which initiate carpogenesis are to be found in the profuse branching of the negatively geotropically oriented bundles of hyphae which are profusely branched and result in the irregular tangled growth.

That many environmental factors are involved in hastening or retarding maturity is well known. Such environmental factors as are usually associated with fruiting in the fungi, and for that matter in other plant groups, are not to be looked upon as initiating factors but rather as indirect factors. Organisms reproduce when they are reproductively mature but the external environment may hasten or retard this maturity.

That change to aerial environment is characteristic of the initiation of carpogenesis is obvious, but when Magnus (1906) states that "Sicherlich ist der Übergang in das Luftmedium sowohl wie die Erschöpfung des

Substrats an Nährstoffen von wesentliche Bedeutung. Ob aber im einzelnen der höhere Sauerstoff—wie niedere Kohlensäure—oder Wasserdampfgehalt wirksam sind, bleibt noch zu entscheiden," he confuses the important question as to the nature of its change in "Stimmung." That this change is due to exhaustion of food supply or other change in the medium has been many times claimed (Klebs, 1904; Magnus, 1906; Munk, 1912; *et al.*) but never decisively proved. I have recently discussed elsewhere (1930a) some of the data under this head and have suggested the possibility that in such fungi as exhibit repeated cyclic changes involving an alternation of vegetative with reproductive mycelium, Liesegang phenomena in the hyphae or in the substrate may be associated with such changes.

The change in "Stimmung" which occurs when the organism is said to be reproductively mature appears to be rektipetiotic, that is, it is caused by internal environmental factors, and these may be caused by changes that occur in the food and other substances after they have traversed a certain distance through the vegetative parts. Klebs (1900) and Magnus (1906) have suggested the possibility that changes occur in food materials which may make of them stimulating substances which initiate the formation of certain organs only after they have traversed a certain distance from the spore. So too, certain chemical substances in the food materials may initiate carpogenesis only after they have traversed a certain distance from the spore or center of inoculation.

In the higher basidiomycetes it is now quite generally held that a substitute sexual act involving the formation of clamp connections and hyphal fusions takes place in the mycelium. In all the cases so far described these processes occur long in advance of the formation of the basidiocarp initials. Is it possible to assume that certain specific "stuffs" are formed at this stage and that these must undergo changes during their passage through the hyphae, making of them substances which initiate carpogenesis? Possibly specific basidiocarp initiating chemical substances are formed by or with the coöperation of the nuclei at particular periods, such as at the time when the binucleated condition first occurs or at certain stages of nuclear division. It may be assumed that since carpogenesis does not begin immediately after the presumed substitute sexual act the hypothetical initiating substances mentioned have to undergo further modification in passing through a certain distance in the hyphae.

There is to date *no evidence that the reported hyphal fusions and clamp connections are associated with the initiation of carpogenesis* and I know of no instance throughout the organic world where the sexual fusion of gametes occurs so long in advance of the initiation of the fruiting stage. The Kniep-Bensaude hypothesis of a substitute sexual act involves a long period of hyphal growth between the hyphal fusions and the initiation of fruiting and it is therefore difficult to accept.

My previously reported observations on hyphal anastomoses indicate

that they have other functions than those associated with sex. The old theory that hyphal anastomoses are a means of equalizing the distribution of food materials may involve something of the concept which Gwynne-Vaughan (1928 and 1930) designated as *nutritive heterothallism*. Gwynne-Vaughan (1930) reported that *Humaria granulata* is heterothallic and that single spore cultures of either the + or the - strain produced oögones which failed to develop into ascocarps. The mycelium and the oögones which it produced were in each strain morphologically alike and obviously female. When the appropriate + and - strains of oögone-producing mycelium were grown together in the same culture there were numerous hyphal fusions and the oögones produced developed into mature ascocarps. This condition the author describes as heterothallism of a type which she tentatively describes as nutritive heterothallism, necessary for reproductive activity but in no way associated with sex.

The two-spored species of *Agaricus* studied is, as stated, homothallic (Lambert, 1929) so that strictly speaking "hybrid vigor" resulting from the crossing of different strains cannot be assumed. Possibly, however, the anastomoses between hyphae which originated from the same spore have some such function involving the equalization of distribution of vitamins or even hormones and are in no way associated with sex.

The Bi-polar Pin-head

The young anlagen when they first become visible to the unaided eye appear globular in form and while in these stages there is no differentiation, a distinct bi-polarity exists. There is the basal region in which there is a preponderance of more or less vertically oriented hyphae and the apical dome-shaped part in which the hyphae are irregularly interwoven. The basidiocarp mycelium in these young stages is loosely interwoven and while, in general, the hyphae grow and branch in all upward directions there is a slightly vertical orientation in the mass. Little tendency to adhesion has been noted in the youngest stages, interhyphal spaces are large and abundant, varying in dimensions between 10 and 20 microns.

The smallest pin-heads studied were about 90 microns in diameter and around 200 microns high and in form were not always globular but round-topped, cylindrical or ovoid. The young bodies may be as in *Cyathus* (Walker, 1920) the modified terminal portion of a strand branch. The tissue is at this stage a prosoplectenchyma and at the base of the pin-head the hyphae are branched in fan-like fashion. This is suggestive of the diminutive forest of hyphae described by Harper (1902) for *Hypochnus* except that the result is a spherical mass instead of a turf or palisade. Above this basal area of profusely branched, erect, and diverging hyphae is the loosely interwoven plectenchyma of irregularly oriented hyphae.

The zone of transition between basidiocarp and strand is a gradual differentiation, the hyphae in the latter are all parallel, then branching

begins, becomes profuse, the hyphae are more loosely aggregated and finally there is the loose prosoplectenchyma of the carpophore (Pl. LII, fig. 1). The hyphae in the strand are always tightly packed together and show a marked tendency to adhesion while in the basidiocarp tissue little adhesion has been noted. The profuse branching and intertwining produces an entangled mass and it is by this means that the hyphae are held together to form the young fruit body.

The marked adhesion between the hyphae in the strand is naturally associated with their functions of storage and transportation. They are highly specialized structures as compared with the basidiocarp initials. The rapid growth and branching of the hyphae results with the factors which initiate the production of the fruiting structure in the globular prosoplectenchyma. These factors (or factor) are similar in a sense to that which comes into play when the slime mould begins to form a stiped sporangium. The plasmodium which has been an inherently unoriented growth and cell multiplication process, becomes aggregative at maturity, definitely oriented movements begin and a centripetal radial symmetry becomes characteristic (Harper, 1929).

The basidiocarp gains rapidly in diameter but only slightly in height. There is a continuous broadening at the base with a lesser development in the apical region so that a round-topped approximately cone-shaped body results. The lower part of the basal region forms an inverted truncated cone as it narrows to its point of origin on the strand (fig. 6).

After the basidiocarp initials form and the stages in cell differentiation begin, organization into the specialized tissues commences.

ORGANOGENESIS AND MORPHOGENESIS OF THE MATURE BASIDIOCARP

The analysis of the processes of tissue differentiation may be conveniently classified under the following heads: Morphogenesis of pin-heads (hyphal morphallaxis, hemi-spherical pads, early morphallactic tissues); tissues emerging from the fundamental prosoplectenchyma by growth movements and cell differentiation; histogenesis of the stipe (early stages, later stages); the pileus initials; the histogenesis of the lamellae (early stages, mature stage); and irregularities in the tissues.

Morphogenesis of Pin-heads

Hyphal Morphallaxis. The basidiocarp represented in figure 3 already shows some differentiation in the hyphae. In the peripheral region where the hyphal cells are exposed to the atmosphere they tend to be of smaller dimensions, tend to branch less, and a greater number of them are parallel to each other and somewhat vertical in their growth directions. The vertical growth directions and smaller diameter of these peripheral hyphae are already indicated in the stage previously described (fig. 1). These hyphae are from the beginning exposed to the external atmosphere and have

consequently undergone physiological changes which render them sufficiently positively thigmotactic to result in their closer aggregation. Their smaller diameter may be a slightly plasmolytic effect caused by the change to aërial environment or shrinkage due to drying out which in some cases may even be the cause of the premature death of many.

The more centrally placed hyphae in the young basidiocarp are exposed to an atmosphere, in the interhyphal spaces, which is continuous with the external atmosphere but is obviously different. If in no other respect, the interhyphal atmosphere will most certainly differ in vapor pressure which will, in all probability, be higher because of the absence of convection currents, the higher carbon dioxid concentration, possible excretions from hyphae, and other causes. This difference alone may bring about chains of inter-related activities causing differences in responsiveness in the centrally located hyphae. That the peripheral hyphae branch less and grow more vertically may be a consequence of the fact that the combined growth of the inner hyphae exerts an outward pressure and because the resistance offered by the apical portion would, on account of the lesser volume, less dense growth, and greater plasticity of younger hyphae, be less than in the radial direction. The factors concerned with the upward growth of inner tissue would be analogous to those described for the vertically elongating young ascocarp of *Sphaerotheca* (Hein, 1927).

That vertical growth and positive thigmotaxis are the determining factors in the responses manifested in the peripheral hyphae is a possibility. All hyphae of the basidiocarp may have an inherent rectipetality or negatively geotropic tendency and only the peripheral ones are sufficiently favorably located to express the inherent vertical tendency. The inner hyphae through crowding and interweaving may present mutual mechanical barriers which prevent the expression of the geotropic response thus causing the indefinite growth directions such as are manifested in the early stages.

The young basidiocarp is on the whole, as stated, generally ovoid in form and smoothly rounded at the apex. In section the general appearance of an .8 millimeter carpophore presents a densely crowded central region, especially near the base which becomes gradually less dense toward the peripheral parts. A comparison of the two regions shows that the hyphae in the dense region are of larger diameters (7 to 8 microns) with few and small interhyphal spaces (Pl. LV, fig. 36), while near the peripheral region the hyphae are of much smaller diameters (4 to 5 microns) and the interhyphal spaces numerous and large (fig. 38). In sectioned and stained material the vacuoles are lined with dark and dense granule-like bodies (Pl. LII, fig. 15). The bodies vary in size and in some vacuoles almost completely cover the inner side of the membrane.

Individual hyphae are by no means uniform and slender as Atkinson (1906) stated but they are very variable in shape, size, staining reaction, and the number of nuclei in the cell. Lack of uniformity, I should say, is

characteristic. Some are rather stout, short to long, gnarled and irregular, while others are short and slender or long and slender with uniform diameters. Stout hyphae vary in diameter between 6 and 8 microns, more slender ones are generally about 5 microns in diameter except in the peripheral zone where about 2 to 5 microns is the more common diameter. The length of the cells will vary between 10 and 60 microns, 30 microns being a common size.

Hemispherical Pads. Not all cells are provided with the hemispherical pads or dumbbell-shaped protoplasmic connections such as have been described by Harper (1902), Nichols (1904), and Levine (1913), and which have since been many times figured, but they do occur on the septa between most cells in every stage of basidiocarp development. In the stages just described the pads tend to be small and very variable in size, staining reaction, and general appearance. Often the pads are extremely minute, pale, and irregularly spherical in form with a thin protoplasmic thread passing through the center of the septum between them (figs. 6 and 23). Generally the pad on one side of a septum is larger than its counterpart on the other side but I can find no correlation between this size difference and the direction of hyphal growth. There may be a large one at the apical end of a cell and a small one in the basal end of the next succeeding cell or vice versa. In a later stage these pads are so numerous, large and conspicuous that they give the first indication of the development of hymenial primordium. Even before such hyphae are very definitely oriented in the vertically downward direction the pads become very large and conspicuously stained with the red of the Flemming triple. In such cells the pads occupy almost the entire lumina at the ends of the cells and except for the thin thread which connects them the two pads are separated from it by a very narrow pale area. The form of the pads varies here as well as in other cells from perfectly spherical to roughly hemispherical.

Early Morphallactic Tissues. Little further internal differentiation takes place in the young basidiocarp just described until it reaches a height and approximate diameter of about one millimeter. Between this stage and the previously described one the peripheral layer becomes more distinct through increase in the number of hyphae and the accumulation of shrunken and dead cells. The innermost zone of dense hyphal development increases in size until it occupies the greater portion of the basidiocarp. Three zones are distinguishable at this stage: the thin outer peripheral layer, a less dense area of uniform width, and finally the central portion just described. In this last mentioned zone a general radial tendency from the center outward may be noted (Pl. LII, fig. 3). There is no evidence of the so-called "couche pileogene" of Fayod (1889) in my sections of these early stages.

From now on the growth of the basidiocarp produces only slight tissue changes. The central area which was at first the denser area becomes slightly less dense probably through hyphal elongation.

The outer zone which consists of the narrow densely interwoven peripheral layer of hyphae (fig. 2a) enclosing the less densely interwoven wider area (fig. 2b) becomes more dense and a new still more dense and narrow zone appears (fig. 5c) between this layer and the inner tissue. This last layer is rather sharply marked by deeply staining hyphae which also show a greater osmiophilic tendency. The new zone (fig. 5c) marks the inner boundary of the pericarp, universal veil, and completely encloses at all points the inner tissues. The dark layer in later stages increases in thickness through differentiation of the surrounding layer and eventually in stages which are about one centimeter in height, it occupies the whole peripheral zone from the outside to the margin of the central tissue (figs. 10 and 15).

The layers just described may possibly arise as a result of partial water loss or exposure to the atmosphere. The cells of these layers are in their origin, diameter of cells, and dimensions of cell walls, no different from those of the central tissue, but it appears that there is a slowing up of the growth processes and in failing to keep up in growth with the more protected enclosed tissue.

Tissues Emerging from the Fundamental Prosoplectenchyma by Growth Movements and Cell Differentiation

When the carpophore reaches a height of about one millimeter the further indications of tissue differentiation occur. The tendency to radial orientation from the center outward to a zone slightly less dense and completely surrounding the carpophore becomes altered. Three-fourths the distance from the base an annular zone of vertical downwardly growing hyphae begins to appear (figs. 3 and 17). These hyphae grow rapidly and profusely in a downward direction, are somewhat terete and continuous with the hyphae of the axial region. There may be traced even in this early stage a continuous line of growth of hyphae from the axial region upward, then a gradual bending radially and eventually vertically downward. This path of hyphal growth becomes more pronounced in a later stage (fig. 7). The annular zone of vertical downwardly growing hyphae now appears to be the most actively growing, initiates and determines the position of the different tissue regions. The axial region of the stage represented in figure five shows increasingly more vertical hyphae until at the stage represented in figure seven nearly all the axial hyphae are arranged vertically. The vertical orientation of the axial region may be the result of an upward pull by the pilear region which is raised by the vertically growing annular area. The upward pull may thus provide the stimulus which initiates further vertical growth of the axial region initiating the development of the stipe.

Histogenesis of the Stipe

Early Stages. The stipe region as well as universal veil and pileus are at once laid down as Fayod (1889) and Atkinson (1906) stated when the

hymenial primordium becomes differentiated. In this early stage the stipe tissue is essentially similar to the pilear tissue, that is, it is an indifferentiated aggregation of irregularly growing and branching hyphae (fig. 4). At about this stage and in succeeding stages may be found growing in the axial region between the older hyphae numerous vertical hyphae which are in other respects (staining reaction, length and diameter of cells, and septation) similar to them. These vertically growing hyphae are most abundant immediately above and below the region of the annular zone and nearest the center of the central axis (figs. 4 and 5). The most active growth of the vertical hyphae appears from their densely aggregated condition to be more abundant below the annular zone than above it and in succeeding stages is almost exclusively below.

The stimulus which initiates the vertical growth may be a mechanical one as is suggested by the general appearance of a median section (fig. 5). The annular zone of primordial hymenium may possibly have an inhibiting effect on the growth of the central tissue contained within its bounds and the latter being still actively growing finds the path of least resistance in vertical directions. Having initiated a vertical direction of growth further hyphal growth in this region will continue in the vertical interhyphal spaces which would obviously offer less mechanical resistance to growth.

By marking with india ink dots in the region below the pileus, I have found in all stages from seven millimeters in diameter up to maturity that after the hymenial veil is broken the most rapid growth is made at and immediately below the zone which is on a line with the margin of the pileus.

In addition to the active vertical growth in the axial region there is a general intercalary growth throughout the stipe as well as the pilear region as shown by the numerous hyphae with dense cytoplasm irregularly located amongst older more vacuolate hyphae. This general intercalary growth results in the formation by spreading apart and disentangling older hyphae, of numerous, large, irregularly shaped but somewhat elongated interhyphal spaces (fig. 13). In buttons five millimeters wide and larger the interhyphal spaces are uniformly distributed throughout all but the hymenial regions. These spaces, no doubt, function as Atkinson (1914) stated, in a respiratory capacity providing for the escape of carbon dioxid and the circulation of oxygen.

The mushroom, since it requires an unusual abundance of oxygen and gives off a large amount of carbon dioxid (Repin, 1897), no doubt requires an abundance of interhyphal spaces for the circulation of these gases.

The irregularly oriented hyphae which make up the greater portion of the stipe region in a young basidiocarp (fig. 5) eventually become the basal region. The region of vertically growing hyphae described above may be regarded as the stipe primordium. From this stage onward there is practically only vertical growth. The increase in stipe diameter is the result of lateral branching from which there is further vertical growth.

When the stipe attains a diameter of about 5 millimeters, that is, when the pileus has attained a diameter of about 4 millimeters, there appears in the axial region at the point where the pilear tissue meets with that of the stipe, a small, triangularly shaped opening. It appears to result from the radial expansion of the pileus from the center outward. That this is a tearing process resulting possibly from the failure of tissues to keep pace in growth with one another is shown by the numerous broken hyphae in the cavity.

The opening which is in early stages an inverted cone shape, equilateral in section, soon becomes isosceles and still later in mature stages a cylindrically-shaped hollow running throughout the length of the stipe. This too may be, like the air spaces, a fortuitous formation for the circulation of gases, and may have been of evolutionary significance to the species.

Later Stages. When the basidiocarps attain a diameter of about one centimeter the pileus becomes more distinctly separated from the stipe and a separation of the two tissues occurs, beginning at the periphery of the stipe and moving progressively inward. Because of the many torn hyphae on both sides of the cleavage it appears that the separation is caused by a pulling apart of the two tissues. The pileus in expanding slightly in radial directions, which are at right angles to the orientation of the stipe hyphae, pulls its tissues away from the latter, resulting in the fissure (Pl. LV, fig. 35a). The fissure in mature stages does not become complete but occupies about half the total area of the stipe in the cross section.

No specialized vascular hyphae are present in the stipe but the hyphal cells are similar to those found in the early stages. Large elliptical interhyphal spaces which appear to be more or less continuous with one another in irregular fashion occur uniformly throughout the tissues. Possibly liquids are conducted through these spaces to the pileus and other parts of the basidiocarp. Fresh living sections when pressed exude considerable quantities of watery fluid and this suggests such a possibility.

The Pileus Initials

The pileus is dome-shaped from the beginning. Before the boundaries of the pileus are laid down by the annular ring the hyphae grow irregularly in every direction as has been described and are similar in their orientation to those in other parts. When the pileus is outlined the previously described vertical hyphae destined to become the stipe form continuous lines curving radially upward then outward and downward to the annular ring giving a fountain-like orientation of the hyphae, which persists up to stages when the pileus is about two centimeters in diameter. The recurved and downwardly growing annular zone of hyphae continue their growth in the same general direction. Continued intercalary growth with branching produces in this zone a crowded condition, the hyphae becoming so tightly packed that few interhyphal spaces remain and a dense palisade of vertical hyphae

is formed. Immediately below the annular hymenial primordium in the fundamental tissue, the hyphae are larger, more vacuolate, and irregular in their growth directions and appear to be old and not actively growing. Between them are large interhyphal spaces and here and there an apparently broken or torn cell (fig. 19). It would appear that the centripetally expanding palisade layer exerts an outward pull resulting in some tearing with consequent very slight destruction of the tissue below it. The elongation which takes place in the axial region brings about a lifting up of the whole cap with further pulling away of the hymenial palisade from the fundamental tissue below. A narrow continuous annular space is thus created by a combination of the raising of the pileus and the centripetal widening of the palisade. With further elongation of the axial region and apparent slowing up of growth in the loose cells below the hymenial palisade the annular cavity enlarges (figs. 7 and 19). From this period onward the cavity becomes broader and deeper with the expansion of the hymenial primordium.

The continued growth with branching of the hyphae at the margin of the pileus with most active growth at the point where hymenial palisade ends causes the hyphae to fill in the annular cavity (figs. 7*a* and 13) as rapidly as it is formed. Figure 8 at *b* shows the incurved hyphae as they tend to grow into the interhyphal spaces and into the annular cavity. The original tissue shown in figure 7*a* is soon left behind by the radially expanding hymenial palisade and the incurved hyphal growth from the pilear margin builds up a new tissue which, as stated, in early stages tends to fill up the annular cavity (fig. 8). It is this new loose tissue which becomes the veil. Where the latter tissue meets the stipe tissue a sharp line of demarcation is formed (fig. 8). The veil hyphae do not intermingle sufficiently with those of the stipe to make a continuous tissue but their different orientations result in the sharp separation. The hyphae in each of the two adjoining tissues, while they grow in various directions, are in general oriented at right angles to each other. The line where they meet remains distinct even in later stages (fig. 13). Some of the hyphae of the veil do enter the interhyphal spaces of the stipe and intermingle somewhat with stipe hyphae but a continuous tissue is never formed. The sharp line of demarcation always remains (fig. 13).

Development of the pileus is in general radially upward and in centrifugal directions. A continuous combination of upward and outward growth results in an extension of tissue surrounding previously formed tissue. There is no evidence in these early stages of intercalary growth in the pilear and subhymenial tissues though presumably some must take place. That pilear development takes place through the addition of tissue by peripheral hyphal growth is shown in prepared sections by the apparently actively growing hyphae at the margins and in growing basidiocarps by markings made with india ink during successive stages of development.

Buttons from five centimeters in diameter on were marked with dots one millimeter apart from the center to the periphery. Larger mushrooms, one centimeter to four and over, were marked with dots two centimeters apart. In stages whose pilei were between five and ten millimeters in diameter the spacing of the dots showed that marginal growth with very little growth toward the center takes place. In larger sizes marginal growth takes place with no perceptible growth in the inner regions, although cytological preparations show that some growth goes on continuously throughout all parts of the pileus. The growth region in mushrooms larger than one centimeter is usually no more than about five millimeters in width.

Histogenesis of the Lamellae

Early Stages. The earliest indication of the hymenial primordia, as mentioned above, is the annular palisade ring of vertically oriented hyphae (figs. 3*d* and 18). Hyphae branch and grow downward from the undifferentiated plectenchyma above the region which is destined to become the hymenium, resulting in a dense hyphal mass with very few and small interhyphal spaces (fig. 17).

This palisade of downward vertically growing hyphae continues progressively outward (fig. 18) forming an ever widening ring. The ring which is at first roughly circular in transverse section (fig. 17) becomes rectangular in later stages as it widens centripetally (fig. 18). In its early stages the base of the ring, the morphologically ventral side of the pileus, is continuous with the fundamental tissue below it, but as the palisade hyphae become more numerous and crowded through intercalary growth, the fundamental tissue eventually becomes separated from it (fig. 18*a*). The sharp separation from the fundament is marked not only by the thin horizontal annular space but also by differences in the hyphae themselves. The palisade hyphae are filled with cytoplasm, show few vacuoles, are in general deeply stained, and all the septa are provided with the often dumbbell-like, hemispherical pads. The hyphae of the fundamental tissue, on the other hand, are abundantly provided with vacuoles and the septa for the most part do not have the pads. When the pads are present, and there are very few, they are extremely small.

The nuclei at this stage as well as in earlier stages are numerous in most cells. A number of twelve or more in a single hyphal cell is not uncommon. Further data concerning the nuclei, however, are still too incomplete to be reported at this time.

The palisade from now on continues to widen and when it attains a width not quite twice that shown in figure 18, about one-half a millimeter or more, an upward arching becomes evident; that is, it tends to buckle or bulge, in a fairly even curve (fig. 19). The annular ring thus becomes concave on the under side and convex on the upper. From general appearance the palisade seems to be mechanically inhibited by the pilear margin

on the outer side and the stipe fundament on the inner. The increase in width through intercalary growth as well as growth at the outer margin results presumably in pressure in each direction causing it to arch upward. The arching pulls the palisade still further away from the fundament, leaving a still wider cavity which is slightly crescent-shaped in section, between the two tissues (figs. 7*a* and 19*a*). The bulk of the growth, always most abundant at the margins as indicated by the india ink markings previously mentioned, is almost entirely in this region. The unfolding at the margins with little growth in the older tissues accounts for the centripetal widening of the pileus.

At about this period a tangential section will show that near the stipe region, rhythmic series of groups of palisade hyphae grow more rapidly and beyond the others. These are the first indications of the gill primordia (fig. 22). Here and there near the stipe these early gill anlagen are continuous with the fundamental tissue below (fig. 27).

The later growth and development of the gills occurs by differentiation from the pilear margin as described by Levine (1922). At the pilear margin is the region of most rapid growth. Here the hyphae are, as stated above, loosely aggregated in the outermost parts (fig. 8*a*) and become gradually more compact by continued intercalary growth until near the cavity a dense palisade is formed which is continuous with the gill rudiments. The gills at the pilear margin are continuous with the fundamental tissue below it.

The earliest indications of the differentiation of the pilear marginal tissue to gill are shown by a short streak (fig. 25*a*) which results from the separation of the hyphae at this point. This streak soon elongates, becomes wider as the gill develops and ultimately becomes a space which is horizontally continuous (figs. 25 and 43) with the interlamellar cavity. This differentiation of pilear marginal tissue into lamellar tissue occurs in all stages later than those following the very young button stages shown in figures 4 to 7. Figures 25, 26, and 43 show successive stages in this pilear marginal differentiation in young mushrooms six, eight, and twelve millimeters in diameter, respectively. The apical part of the gill, the region of growth attached to the fundamental tissue, eventually separates from it, becomes rounded by further hyphal growth, and then resembles older portions and such parts of the gill as are developed from the palisade into a pre-lamellar cavity.

The lamellar tissue at the pilear margin is curved vertically and is nearly parallel to the outer margin of the pileus (figs. 8 and 13). As the pileus widens through marginal growth this lamellar tissue gradually straightens out so that its hyphae become parallel to the previously formed ones in the older portions of the gills. It is a process of unfolding possibly analogous to the unfolding of a leaf in higher plants.

The marginal growth results in an accumulation of fundamental tissue,

b in figure 8, which, mechanically inhibited by the stipe, results in a slightly upward thrust causing the marginal curve of the pileus and, therefore, the curve in the lamellae (*a* in figure 6) to straighten out. The straightening out, with expansion of the pileus, while it may at least partly be caused by the upward and outward push from the fundamental tissue as described, is without doubt also assisted in this process by continued intercalary growth in the palisade as well as in the subhymenial tissues causing a general distension, as must be the case also in such carpophores in which there is no veil.

Occasionally there are young basidiocarps in which the curve in the palisade is unusually great and the part near the stipe is sometimes at an acute angle and almost vertical to the stipe (fig. 34) but such figures are unusual, and it is not certain that they ultimately develop into the typical basidiocarp or even reach maturity.

Most of the young gill primordia in the earliest stages, however, arise as radial ridges consisting of groups of downwardly growing palisade hyphae. That in general a very narrow slit-like annular cavity precedes the first indications of the gill primordia and that such cases as figured in 29 are not the typical mode of origin for the gill appears evident from my sections. Certainly there is in general a smooth palisade and annular cavity below it which precede the formation of the gill primordia.

Figure 20 is a tangential section through the hymenial region midway between stipe and pilear margin at a stage similar to the one whose radial section is represented in figure 19. A palisade, irregular on the morphologically ventral side but free from the fundamental tissue, is represented. No suggestion of radial ridges is shown and a cavity is just beginning to appear. Figure 21 is a slightly older stage of a similar tangential section. Here the palisade is perfectly smooth, entirely free from the fundament below, with a rather wide cavity between. Similar stages to these have been sectioned at right angles and obliquely to the axis of the sporophore. Figures 28 to 33 are selected from successive sections from a single sporophore cut slightly oblique but nearly at right angles to the axis of the stipe. The sections represented are in the hymenial region and show the various parts of the annular ring from top to base. Figure 28 shows the dense palisade above the cavity. Figure 29 is lower and shows part of the cavity. Figure 31 is still further down. No radial ridges are shown here. Neither are there the dense hyphal aggregates which should occur at this stage if the ridges precede the formation of the cavity. Figures 32 and 33 are still further below the above. In figure 33 part of the palisade is shown in the lower half of the figure. The upper half shows the fundamental tissue.

Near the stipe region (fig. 16), however, and often on the outer margin such appearances as represented in figure 27 are more common in early stages. Figures 9, 16, 14, 12, and 11 represent typical tangential sections

from various regions of a single carpophore taken at intervals in serial order from the margin almost to the stipe region. Figure 9 is close to the margin, the active growing region, and shows the dense palisade with fundamental tissue below it and a thin streak between, the first indication of the annular gill cavity. At figure 16 the palisade with its smooth basal margin and cavity formed by the arching of the palisade is shown. There is no indication of the gill primordia at this region. Figure 14 is nearer the stipe and here irregular connections between palisade and fundament occur. This connecting tissue appears as though it is being torn away (fig. 27) by the arching of the palisade. Excepting in the stipe region and pilear margin in later stages the gills clearly arise as radial plates growing downward into a prelamellar cavity.

No evidence of factors which initiate the development of gill primordia has been found. There do not appear to be specialized cells differentiated either in form or content as might be indicated by staining reactions which develop more rapidly than others to form the gills but all the palisade cells are alike in appearance. When the gill primordia begin to develop, the tissue between them which eventually becomes the base of the interlamellar space acquires a slightly compressed appearance. The gill hyphal ends are rounded at the apex, the terminal cells being clavate in form (figs. 22 and 24) while the ultimate hyphal cells between the young gill anlagen are often more rectangular and at times slightly terete (fig. 24).

Figure 24 is a stage slightly later than that shown in figure 22. The gill primordium has grown downward and the trama is becoming differentiated by the appearance of elongated cells. The young tramal cells are long and slender and obviously grow a little more rapidly than the surrounding hyphal cells. This results in pushing the latter cells to each side giving a beautiful symmetrical pattern of downwardly radially growing hyphae (fig. 24). The interlamellar spaces in this early stage are broad and roughly triangular with rounded corners. As the young gills continue to grow downward they become somewhat broader, lessening the space between them. I do not find that any of the gill primordia fail to develop into gills by possible crowding. They are apparently not crowded out as might be supposed from the limited space and the great width which they and the interlamellar cavities eventually attain. From the center of one gill trama to the center of the adjoining gill the distance is increased about one-half the original distance in basidiocarps whose pilei have developed to about four millimeters. Since there is no hyphal swelling and interhyphal spaces are no more abundant the increased distance can only be accounted for by intercalary hyphal growth.

The lamellae continue to grow downward and increase slightly in width. The outermost hyphae are continuously crowded to each side and are eventually oriented near their ends at right angles to the tramal hyphae (fig. 23). The end cells soon grow far enough outward to touch the corre-

sponding cells of the adjoining lamella. Septation occurs with frequency between the tramal region and the terminal cells (fig. 23) so that numerous short cells are formed in later stages. The width of the lamellae up to this stage is around 65 microns. This width is attained by the basidiocarp almost immediately after the gill has begun to grow downward when the pileus has reached a diameter of three or four millimeters and the depth of the gill is about 50 to 60 microns.

Later Stages. When the basidiocarps reach a diameter of about one centimeter the lamellae have a width of about 110 microns. The tramal hyphae do not increase the width and remain a constant number, about 12 or 14 cells in transections. The increased width of the gills is caused by the elongated clavate cells which become the basidia and by the interpolation of large air pockets.

The tramal hyphae consist of elongated cells and no further septation appears to occur in them after they are once formed. The cells in length measure around 50 microns and in diameter from 6 to 15 microns. Sections cut at right angles to the plane of growth show closely packed cells of various shapes and diameters (fig. 36).

Interhyphal spaces in the cross section are very irregular in shape, size, and distribution (fig. 37). While no cells of extremely large diameters such as are found in the rhizomorphs (Hein, 1930b) are present many have diameters which are considerably wider than the typical hyphal diameters. No evidence of fusions has been found in these cells and they probably owe their slightly increased diameter to expansion.

Studies on the morphogenesis of the basidia and spores are under way and are to be reported at a later date.

Irregularities in Tissues

The form of the basidiocarp as a whole is subject to considerable variation in all stages and while many may be genetic it appears that environment plays an important part in the determination of the form. The stages as described I regard as typical simple because they occur in great numbers and all radical variations from those described are comparatively rare. Interesting abnormalities and malformations caused by injuries and crowded conditions on the other hand are very common and the causes of them are, in most cases, obvious.

In poorly ventilated houses it frequently happens that tall and spindly mushrooms develop. French growers (Repin, 1897) speak of suffocating mushrooms. Possibly these spindly forms result from insufficiency of oxygen and accumulation of carbon dioxide.

Very often young buttons in stages 5 mm. to 10 or more mm., instead of having the typical broad basal region, will grow up in the reverse proportion with narrow stipe and broad pileus. Such mushrooms, as I have observed them, rarely develop to a very large size and often succumb to

parasitic diseases while others in the same bed remain comparatively immune. Growers ascribe this condition to insufficient ventilation.

Here and there in a bed there sometimes appear sporadically unusually large mushrooms with immense stipes. This condition is said to be due to excessive moisture in the beds. Such basidiocarps frequently develop deep fissures which indicate high turgor.

Basidiocarps without veil or partially developed veil sometimes appear as an epidemic. I was of the opinion that this condition may have been the result of parasitic organisms but cytological sections failed to reveal either bacteria or fungi in the region.

In such basidiocarps there usually are vestiges of a veil at the pilear margin. More commonly there is a partially developed veil. The gills remain short and show infinite variations in depth, width, and form. Frequently they are fused with each other and wrinkled. Often there are areas in which no gills are developed and occasionally the whole hymenial surface will show no trace of gill development. The pileus on the other hand will be well developed and thicker in section than the typical. Otherwise such basidiocarps are symmetrical in form, show no discolorations, and are marketable.

The most common variations occur in the lamellae. The lamellae frequently grow so closely together that they become fused sometimes at the base, sometimes at the region of growth (fig. 39). Figure 39 shows two young gills in tangential section which fused at an early stage in their development, separated, and fused again. Such cases are not uncommon and probably are the result of crowded conditions in the hymenium.

Cross sections show that the lamellae frequently fuse both near the stipe region and at the periphery. Figure 41 shows two gills fused near the stipe region which have later separated. Figure 44 is a tangential section from close to the stipe region. The bottom of the gill, having grown more rapidly than the annular cavity, has come in contact with it and the hyphae are diverted from the typical downward direction. Such cases too are not uncommon in this region.

DISCUSSION

The basidiocarp in the beginning stage consists, as has been described, of a tangled mass of interweaving branching hyphae which are in appearance similar to those of the substrate. From the comparatively abundant and more or less uniform distribution of the interhyphal spaces it appears that the tendency to adhesion is far less marked in the mycelium of the sporophore than that in the substrate. When the adjoining lateral walls of two or more hyphae are in contact with each other it is never for more than a very short distance, indicating further a lack of adhesive properties. The mass of loosely interwoven hyphae is held together by its entangled condition. Further intercalary growth with branching results in the denser plectenchyma found in somewhat older basidiocarps. That the young basidio-

carp grows upward from the substrate suggests a geotropic response and the experiments of Brefeld (1877) and Buller (1905 and 1909) showed that some fleshy fungi including the common mushroom are negatively geotropic; and yet since individual hyphae are oriented in every possible direction and young basidiocarps are frequently crowded so that their axes are at wide angles from the vertical, gravitational responsiveness in the early stages is evidently weak. That the axis of young basidiocarps when uninhibited by mechanical barriers is vertical to the substrate may be partly or entirely a response to the stimuli which initiate carpogenesis. Perfect and symmetrical mature basidiocarps whose axes are at various angles to the horizontal substrate are common in the beds.

The form-determining factors involved in shaping the successive stages during development appear to be largely mechanical processes. The emphytic (Wodehouse, 1929) cylindrical form of the hypha undergoes little change except in the mature tramal tissue where mutual contact and pressure relations result in irregularly shaped elongated cells. The region which gives place to the palisade primordium may offer a lesser mechanical resistance to invading growth, and this annular group of hyphae initiated by some stimulus, possibly such as has been suggested by Magnus and Klebs (1900), grows downwardly following in the path of least resistance.

The reasons why the locus of the annular ring of hyphae so initiated should be in this particular part of the young basidiocarp are obscure but may be due to the accident of position.

The formation of the ring of vertical hyphae may be the initial stimulus for the development of all later formed tissues. Having once begun to grow these hyphae may set in motion a chain of interrelated physical and chemical stimuli. From the annular ring of hyphae there may soon be traced in median longitudinal sections a continuous line of hyphae forming a curve to the stipe. The ring may have slightly released the possible tensions existing in the hyphae adjoining immediately behind the tips and this release in tension may then gradually spread centripetally backwards at a uniform distance from the periphery of the pileus to the center, ultimately meeting at a point, the axis of the basidiocarp. At this point the release in tension would be many times redoubled and result in the initiation of the stipe region. The continuation of this process leads to enlargement of the tissues so initiated. The annular zone of vertical hyphae increases in diameter largely by the peripheral addition of similarly oriented hyphae and by intercalary growth. The expanding ring causes the pileus to become broader and the fundamental tissue below the hymenial region, which shows some evidence of having become slightly stretched, is perhaps on that account retarded in its growth so that it fails to keep pace with the upper parts. This results in the narrow diameter of the basidiocarp at the zone which separates pileus from stipe. The zone may be regarded, therefore, as initiated through slight traumatic stimuli caused by the cylindrical form and can nearly always be identified.

The various tissues formed appear to become differentiated through differences in rate of hyphal growth, branching, and multiplication. While rate of hyphal growth may in part be determined by the position in the basidiocarp and be, therefore, governed by such factors as mechanical barriers, oxygen content of the interhyphal spaces, accumulation of carbon dioxide, and other growth-inhibiting or accelerating factors, the distance from the food supply may also be involved. A possibility to be considered is that chemical substances in the food materials become altered in their composition during the passage from cell to cell and may call forth a different morphological response in the successive parts through which they pass. They may therefore be regarded as specific form-determining stuffs. Possibly such changes occur in the mycelium of the substrate and in combination with a complex of environmental conditions together initiate fruit production. Klebs (1900) statement that "die Nahrung beim durchgang der älteren Mycelteile verändert sein muss" supports such a possibility. Similar suggestions are contained in the statements of Magnus (1906): "Es ist möglich dass die Hyphen durch ihre innere Lage irgendwie dazu geführt werden, die feineren späterhin das Hymenium erzeugenden Hyphen zu bilden. Man könnte z.B. denken, dass eine gewisse Entfernung vom Nährsubstrat, ebenso wie eine gewisse Entfernung von der freien Oberfläche der Anlage in diesem embryonalen Zustand als reisanslösend für die Differenzierung wirksam sind." "Hypothetische Möglichkeiten sollten nur zeigen dass auch ohne Befruchtungsvorgang sehr wohl die Differenzierung spezifisch Hymenium produzierende Hyphen an einer bestimmten Stelle der Entwicklung erklärbar ist."

The first indications of tissue differentiation occur as stated above in the region destined to become the hymenium. Here for the first time the hyphae show definite growth orientations which are downward. The downward growth may be the result of the general radial directions which have been observed in the upper parts of the pilear regions. It has been shown that most of the growth in the pileus is in the outermost regions of the dome-shaped portion and possibly these parts may for a time at least act as a barrier which tends to inhibit the growth of the hyphae enclosed by it. In this sense the inhibiting layer is analogous as a morphogenetic factor to the perithecium of *Sphaerotheca castagnei* (Hein, 1927). In the latter I have suggested the possibility that the perithecium is a mechanical barrier against which the ascogone during its growth period is bent in two different directions.

The tissue destined to become the fundamental tissue below the hymenium and ultimately the veil consists of irregularly oriented hyphae in the earliest stages but as the margin of the pileus expands radially the hyphae appear to become slightly stretched, more loosely aggregated, and oriented generally radially from the stipe like the supporting ribs of an umbrella. The rapid radial expansion of the margin of the pileus appears thus to be the principal factor involved.

ORIGIN OF THE LAMELLAE

That there is a distinct cavity formed before there is even a suggestion of the radial ridges which later become the gills is evident from tangential sections in very early stages. A ring of palisade which shows no ridges and is not connected with the fundamental tissue below may be easily identified in certain stages of young buttons. In stages immediately preceding this the palisade is continuous with the tissue below it. When the separation between palisade and fundamental tissue occurs the former shows in sections a somewhat ragged outline as indicated in figures 19 and 20 but this soon becomes, as has been described above, fairly smooth (fig. 21). Sometime later when the thin cleavage line (fig. 19*a*) has widened to form a distinct cavity, the radial ridges appear, but not before. That there are, however, irregular ridges and radial plates of hyphae continuous with the pileus and fundamental tissue, close to the stipe region and in the region near the pilear margin is shown in many of the sections studied. Such appearances may be present even when the hymenial area between them is separated from the tissue below by a wide cavity. The hymenium at this stage is smooth, and the hyphae are terete or rounded at the ends and show no evidence of having possibly been torn away from the tissue below. For these marginal regions my observations confirm those of Levine but for the regions between the inner and outer margin of the hymenium I find that the gill ridges grow into a pre-lamellar cavity.

Stages as late as those shown in Levine's figures 52 and 53 (1922) are well along in their development and the tramal tissues shown are here and there in contact with the tissue below, but are not actually continuous with it. Levine's figures show the fan-like orientation of the terminal hyphae of the lamellae and are plainly growing downward against the fundamental tissue below it if not into an open cavity. Certainly the tramal hyphae even in Levine's figures do not appear to be differentiated from a continuous tissue.

The cavity mentioned is very narrow in the early stages of the basidiocarp and slight changes in the external environment may cause the hyphae to expand or contract with corresponding changes in the interhyphal spaces and this may be associated with differences in the size and curvature of the prelamellar cavity. Then, too, differences in the rate of growth between the hymenial hyphae and the hyphae of the fundamental tissue would result in variations in the depth of the cavity.

Studies on the morphogenesis of the basidium and spores as well as studies on the nuclear history are still too incomplete to be reported at this time.

The writer is deeply indebted to Dr. R. A. Harper for his unfailing interest, for many helpful suggestions, and for encouragement.

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EXPLANATION OF PLATES

The figures were drawn with the aid of the Abbe camera lucida. Figures 1, 6, 22, 25, 26, and 34 are enlarged approximately 250 times; figures 2 to 5, 7 to 12, 13, 14, 16, 17, 28 to 33, 100 times; figure 15, 2000 times; figures 36 and 38, 1000 times; figures 18 to 21, 23, 24, and 37, 350 times; figures 39 to 41, 43, and 44 are enlarged 200 times.

PLATE LII

Figures 1 to 7 are in median sections.

FIG. 1. Very young stage just emerging from the substrate. The mycelium forms an entangled mass of irregularly oriented hyphae.

FIG. 2. One-half millimeter button showing three areas barely distinguishable in the figure of hyphal layers of varying density.

FIG. 3. Later stage. *a*, peripheral layer 2-4 cells in thickness. *b*, loose plectenchyma. *c*, denser plectenchyma.

FIG. 4. The annular zone of vertically oriented hyphae just beginning to appear.

FIG. 5. Later stage one-half mm. button. At *a* a very thin layer of slender hyphae surrounds a thick layer of hyphae of larger diameter which extends to the layer *c*. The thin layer at *c* consists of densely aggregated hyphae which are highly osmiophilic.

FIG. 6. Stage immediately following the one represented in figure 1. No differentiation of tissues has occurred. The hyphae are still indefinitely oriented in all directions.

FIG. 7. Slightly later than 5. The prelamellar cavity and stipe region are formed.

FIG. 8. Enlargement of stage similar to figure 7 in radial section.

FIGS. 9, 16, 14, 12, and 11 are successive tangential sections showing radial ridges near the stipe region, and smooth palisade between margin of pileus and stipe.

FIG. 10. Tangential section which shows dense palisade and radial ridges near the stipe region in an older button.

FIG. 13. A still older six-millimeter button showing the cavity in the stipe near the pilear tissues. The larger cavities throughout both stipe and pileus are plainly visible.

FIG. 15. Hyphae from the stipe tissue of a young button showing the densely stained bodies lining the vacuoles.

PLATE LIII

FIG. 17. Radial section similar to 5*d* but enlarged. The hymenial primordial tissue shows the dumbbell shaped protoplasmic connecting pads. The latter are not present in the surrounding regions.

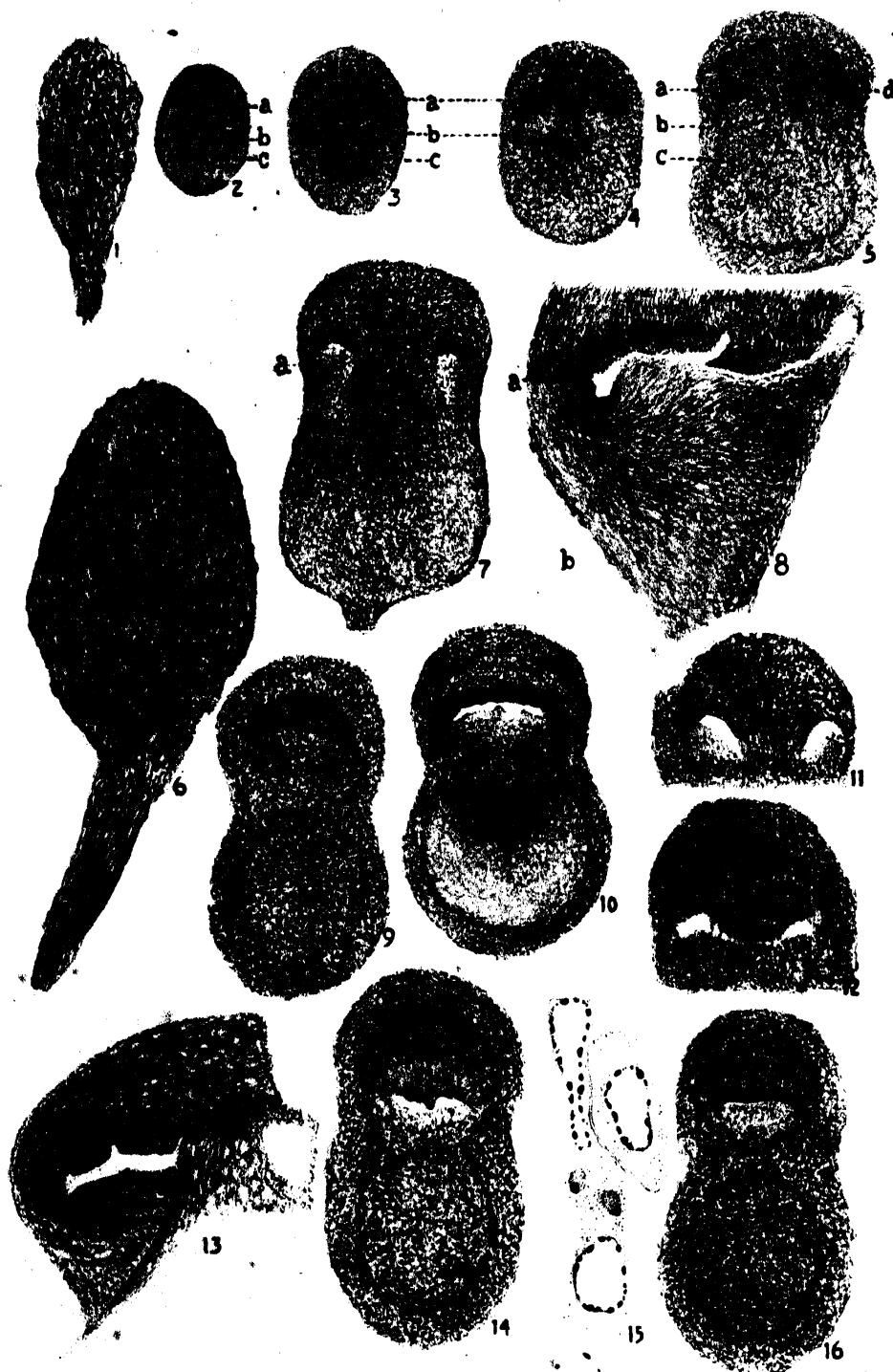
FIG. 18. Later stage also in radial section showing distinct separation between fundamental tissue and hymenial primordium. The hyphae in the latter are plainly growing downward.

FIG. 19. Still later stage. The annular cavity has begun to appear and a dense palisade is formed above it.

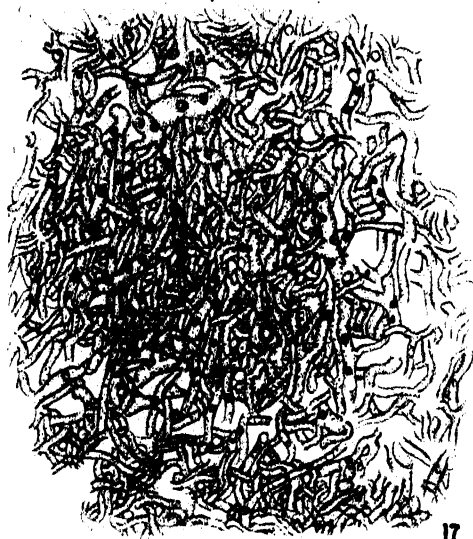
FIG. 20. Similar stage to 19 but in tangential section. No gill primordia are shown but the palisade has a ragged margin.

FIG. 21. Later than 20. The palisade is now smooth and very dense; no gill primordia are present, and a fairly wide annular cavity is shown.

FIG. 22. A single gill primordium from the tissue midway between stipe and pilear regions.



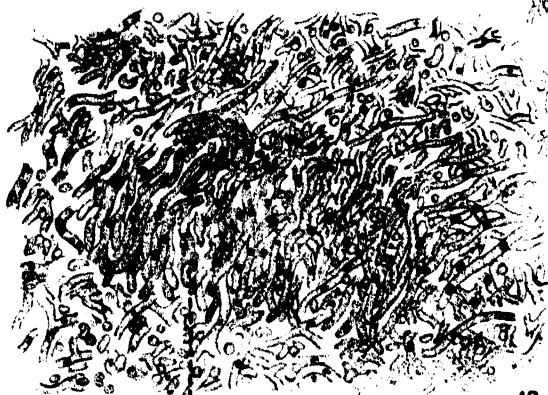
HEIN: AGARICUS



17



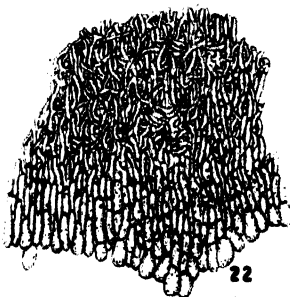
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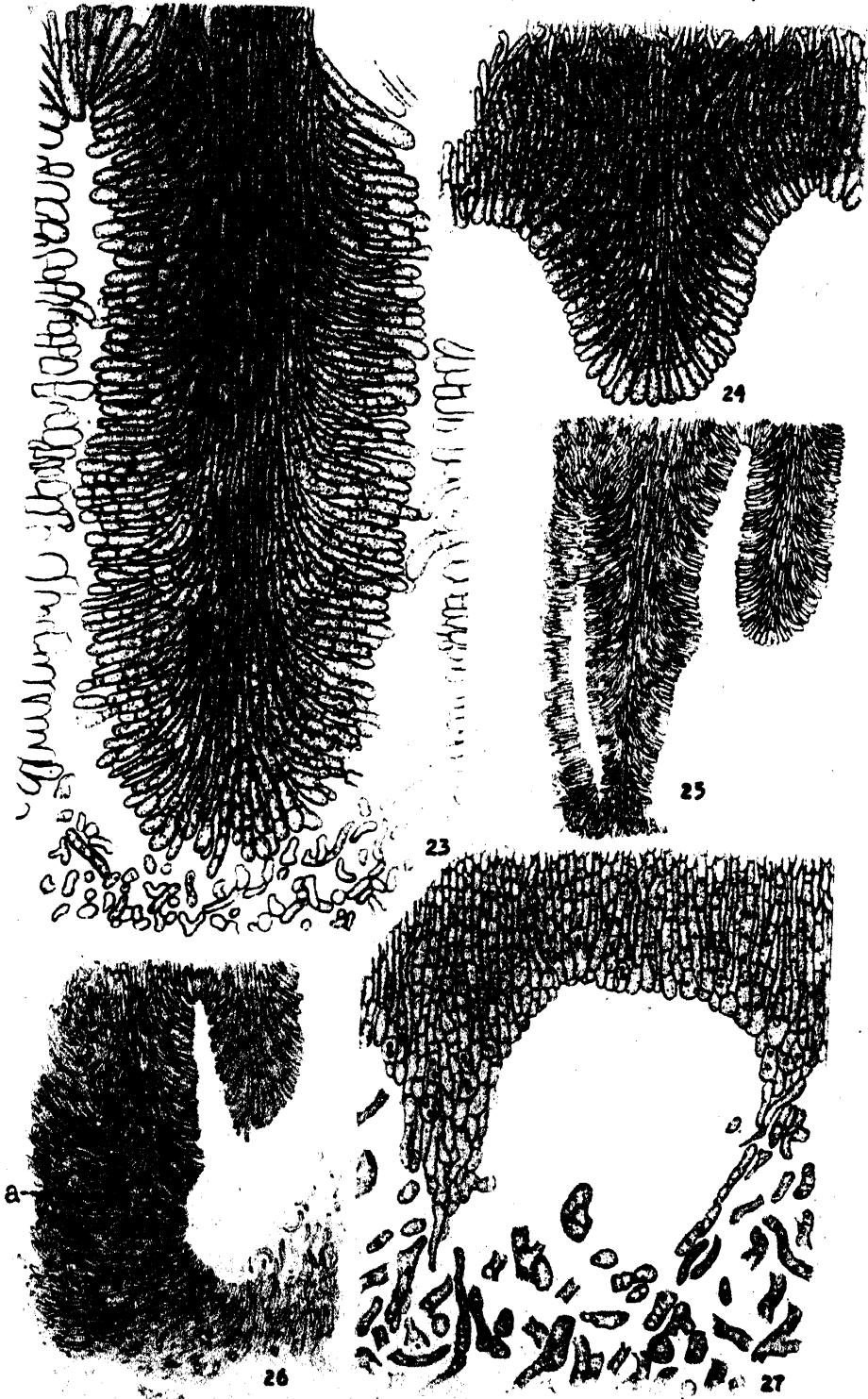
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19



HEBELIA AGARICUS

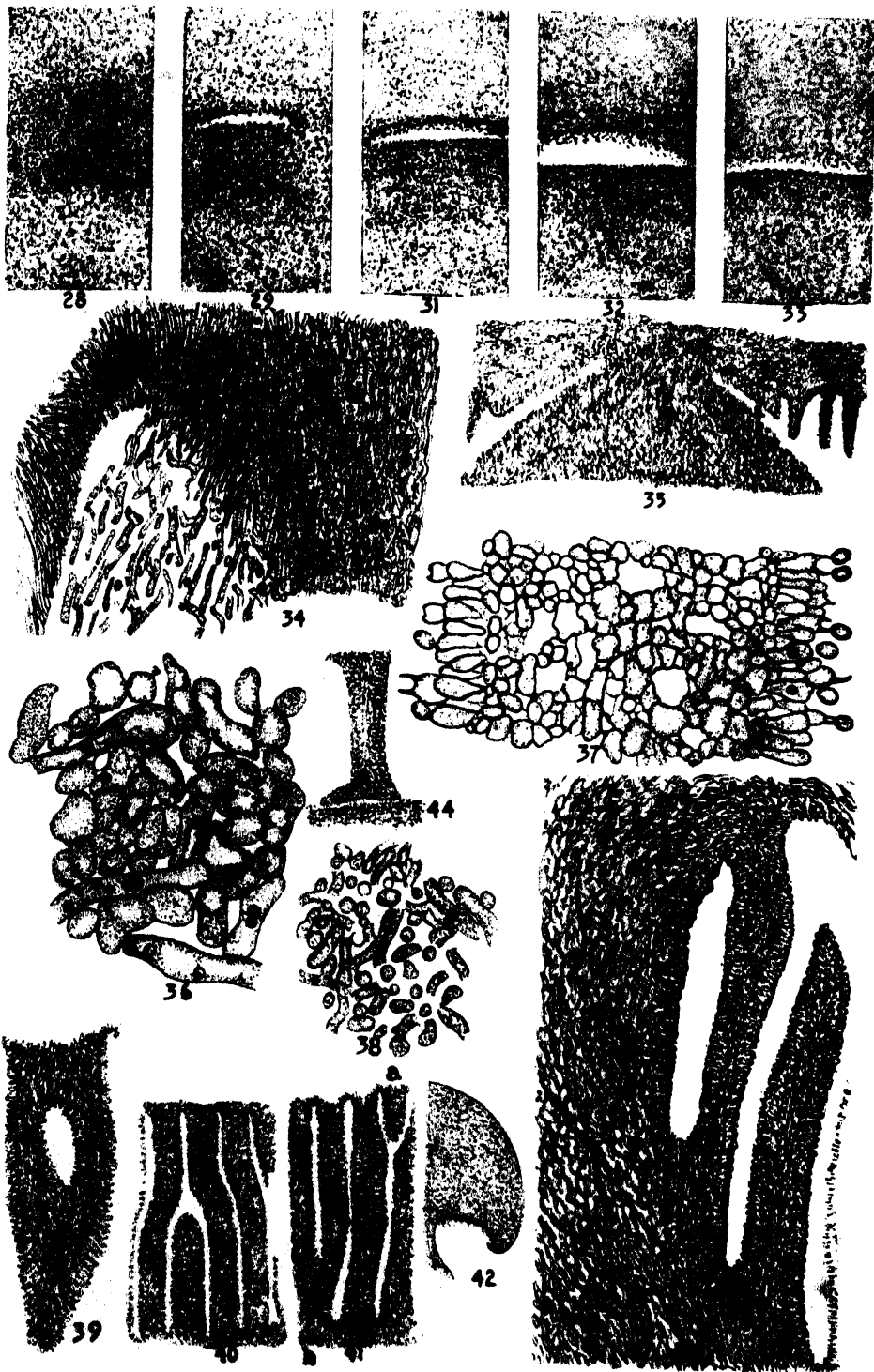


PLATE LIV

FIG. 23. Young lamella in tangential section showing orientation of the hyphal cells. Rapid growth at this stage almost completely obliterate interlamellar and annular cavities.

FIG. 24. Stage preceding 23. The fan-like orientation of the hyphal cells which continue at the apex in all later stages is conspicuous.

FIG. 25. Tangential section. The lamella at the pilear margin is continuous with the fundamental tissue below but later separates.

FIG. 26. An earlier stage of a similar section to 25. The interlamellar cavity is just beginning to appear at *a*.

FIG. 27. Lamellar tissue near the stipe region in tangential section.

PLATE LV

FIGS. 28 to 33. Successive sections of three-millimeter button in transverse sections through pileus at the annular ring. The sections are slightly oblique and show in figure 28 the dense palisade above the cavity; and in figure 29, *a*, part of the cavity is semi-lunar in outline because of the oblique sectioning. Figures 31 and 32 are successively still further down and show increasing amounts of the cavity. The palisade in figures 31, 32, and 33 shows no ridges or dense hyphal aggregates but is perfectly smooth. In figure 33 the fundamental tissue is shown in the upper part of the figure and the palisade near the stipe in the lower half.

FIG. 34. Radial section from a button in which the palisade has become almost vertically oriented. An atypical sporophore.

FIG. 35. Eight-millimeter button. Median tangential section from region at the juncture between stipe and pileus, midway between margin of the stipe and central axis.

FIG. 36. Plectenchyma from the inner basal region of one millimeter button. The hyphae are of wide diameter and closely packed as compared with those near the peripheral regions.

FIG. 37. Transection of mature lamella showing large interhyphal spaces and irregularly shaped tramal hyphae which contain little cytoplasm.

FIG. 38. Plectenchyma from the peripheral regions of the stipe tissue in one millimeter button. The hyphae are narrow in diameter and loosely aggregated when compared with those in figure 37.

FIG. 39. Two lamellae in tangential section which have fused at the base, separated, and fused again at the apex.

FIG. 40. Transection of lamellae, a secondary lamella growing between two older primary lamellae.

FIG. 41. Transection of lamellae showing secondary lamella at *a* just beginning to grow between two older primary lamellae. At *b* two lamellae are fused near the stipe region and later separate.

FIG. 42. Radial section of basidiocarp with hypertrophied hymenium. The gills are short and irregular and have developed no basidia. No veil tissue is present.

FIG. 43. Slightly oblique radial section from the margin of the pileus showing lamellae in this region continuous with the fundamental tissue.

THE CORM AND CONTRACTILE ROOTS OF *BRODIAEA LACTEA*¹

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INTRODUCTION

Brodiaea lactea (Lindl.) Wats. (*B. hyacinthina* var. *lactea* Baker; *Hookera hyacinthina* (Lindl.) Kuntz.) is found in low meadows from California to British Columbia. It produces a solid corm which varies from 7 to 50 mm. in diameter and is covered with a very heavily lignified sheath. The flower stalk, which is 30–80 cm. tall, bears numerous white, open-campanulate flowers in a several-bracted umbel. The mature corm carries two linear-lanceolate leaves which are 5–8 mm. broad and somewhat shorter than the flower stalk. Like many of the cormous monocotyledons, it produces a new corm each year immediately above the old one. Offsets, provided with contractile roots, are also produced in the axils of the leaves.

The contractile roots of these offsets have proved of such interest that in this study emphasis has been placed upon their development. The consideration of the corm has been limited to a rather general survey of its seasonal development.

HISTORICAL SUMMARY

Geiger (5) gives a *résumé* of the development of our present conception of a corm. She states that a corm may be considered to be a vertical modified stem formed by the enlargement of the basal axis of the current year's growth.

Arber (1) describes the inward and outward curvature of the vascular bundles as found in flattened corms, using *Brodiaea congesta* Sm. as a type. The bundles of *B. lactea* are similarly arranged.

Rimbach (18) briefly summarizes the life history of *B. capitata* Benth. Four lateral cormlets are produced which are supported on stalks 1 cm. in length. The stalks wither away the first season, freeing the corms. At the start of the next season a few thin nutritive roots are produced from the lower portion of the offset. Then a fleshy contractile root develops which draws the cormlet out of its protecting sheath and away from the parent corm. Thus in this case the contractile roots prevent the crowding of the corms and do not tend to lower them as is usually the case.

¹ Contribution No. 27 from the Botany Department of the State College of Washington.

According to Scott (20) the knowledge of contractile roots and their influence on the depth of bulbs and tubers has been at hand at least since the time of Nehemiah Grew. In his work on the anatomy of plants, published in 1682, Grew states that the visible cause of the descent of tubers into the soil is the presence of "String-roots," which, "descending themselves directly into the ground like so many ropes, lug the trunk after them."

With reference to contractile roots in general, Arber (1) states that "It seems as though plants which perennate by means of subterranean organs, are characterized, at maturity, by a certain definitive depth position in the soil, which is reached, either by a downward growth of the stem itself, or by the pull of . . . contractile roots . . ." This type of root is found in both the monocotyledons and dicotyledons but is much more common among the former group.

De Vries (4) apparently introduced the first experimental evidence regarding the contraction of roots. He investigated the roots of eighteen different species of plants, mostly dicotyledons, studying the effects of cultivating them in water and of exposing strips of the root, different tissues, and cells to the influence of air, water, and salt solutions. De Vries ascertained that with the absorption of water the living parenchyma cells of the root increase in width and decrease in length. He distinguishes two types of contraction, the gradual process as it occurs in roots growing in nature and the relatively rapid contraction upon the absorption of water. The former is not a reversible process while the contraction brought about by the absorption of water is, to a large extent, reversed by dehydration. With regard to these distinct processes, de Vries says, "Die allmähliche Kontraktion muss als eine Wachstumserscheinung betrachtet werden; die rasche Kontraktion beruht auf einer Aenderung des Turgors." Berckemeyer (2) has recently shown that a differential growth of secondary tissues is the cause of root contraction in certain umbellifers.

Rimbach (7-18) has presented the largest mass of data regarding this problem. He confirms the findings of de Vries regarding dicotyledons and describes the process as it occurs in monocotyledons. In general it proceeds as follows: Soon after the period of active elongation in a section of the root the active cells of the inner cortex undergo a change in form, decreasing in length and increasing in their radial and tangential measurements. The epidermis, exodermis, and a few layers of cells bordering on the latter are entirely passive during this change. The outermost layers of the active cortical cells collapse after a time and become compressed by the expanding inner cells and gradually form a widening zone immediately inside of the passive outer cortex. This passive tissue is lifted in large wrinkles over the surface of the contracted region. The radial-longitudinal walls of the endodermis and hypodermis become undulated during the shortening. The central axis is passively contracted by these changes, usually remaining

straight in the monocotyledons and becoming distorted in the dicotyledons.

Rimbach (18) observed that in *B. capitata*, however, the central strand is undulated and spiraled in the shortened regions of its contractile roots. With regard to this he states that "In this case it seems to be not so much a necessary consequence of the shortening itself, as a consequence of the irregular manner in which the active cortical cells change their form. While in other roots the cortex behaves equally all around the central cylinder, in this case the radial elongation of the cells seems to take place now on one, now on the other side of the root, and the volume of the cortex increases alternately on different sides of the strand. . . . No zone of collapsed cortical cells, however, forms."

Church (3) considered the contractile roots of *Cooperia Drummondii* Herb. She found the same regions of active, turgid tissue enclosed by passive, contracted tissue, but did not find that the cortical cells were shortened. The stele remains straight in the contractile root of this species. In conclusion, Church states that "It seems safe to accept these facts: (1) roots do shorten; (2) the parenchymatous tissues of the root are the seat of this activity; (3) the cork and vascular trace are passive; (4) the cork is ultimately crushed; (5) there is a region where one can see wrinkles and measure shortening, a region where no wrinklings are visible yet where one can measure shortening, and an unchanged region (Rimbach); (6) in dicotyledons the trace becomes visibly curved inward and outward in a wavy fashion, while in monocotyledons the vascular bundles remain practically straight (de Vries)."

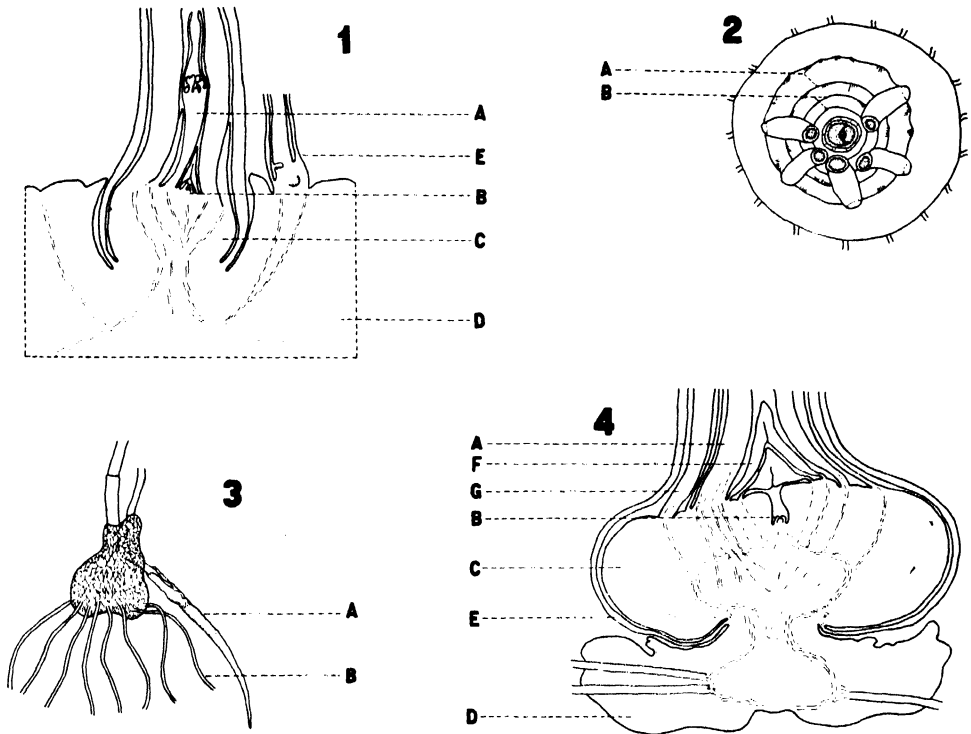
Arber (1) considered the question of root contraction in *Hypoxis setosa* Baker and found practically the same regions of turgid and crushed cells as did Rimbach and Church. She does not accept Rimbach's theory of the contraction and, with regard to this, says, "I am wholly unable to understand how the wrinkling comes about, for it seems to me that the elements of the unwrinkled inner cortex have increased proportionately more in length than have those of the sclerized outermost layer of the root, and yet, instead of being disrupted, the whole of this outer layer is wrinkled—that is to say, it behaves as if it had increased more in length than the inner tissues. . . . I can detect no such shortening as that postulated by Rimbach."

Gravis (6) is the most recent worker to offer evidence as to the probable cause of contraction in monocotyledonous roots. His studies were made on the contractile roots of *Crinum capense* Herb. In this case the stele becomes strongly sinuous and there is the same zone of crushed cells of the outer cortex as found by previous workers. His illustrations indicate that there is an actual shortening in length and an increase in depth and breadth of the inner cortical cells. Gravis also states that during contraction the spiral and annular thickenings on the tracheal tubes are brought closer together. As an explanation he advances the possibility of a phenomenon

inverse to intussusception; that is, a dissolution by the protoplasm of a part of the cell membrane, the displacement of the dissolved substance, and its utilization in a new direction of growth. The small living cells surrounding the tracheal tubes perform this function for the tubes, since the latter contain no protoplasm, accounting for the closing together of the rings during the contraction.

THE CORM

At the time of flowering, June or July, the corm of *B. lactea* exhibits two foliar leaves, a flower stalk in the axil of the second (inner) leaf, and a small vegetative bud sunken in the center of the corm protected by two cataphylls (text fig. 4). The corm is enclosed by a sheath extending from the "neck,"



TEXT FIG. 1. Semi-diagrammatic section through central portion of corm as it appears at end of fall period of growth: *A*, young flower umbel; *B*, vegetative bud of new corm; *C*, new corm which will mature during the spring growing season (see fig. 4, *C*); *D*, corm of preceding season; *E*, offset borne in axil of inner cataphyll, showing rudiment of its contractile root. $\times 2.8$. FIG. 2. Top view of mature corm as it appears in early spring, showing five offsets at node of outer foliar leaf: *A*, nodes of foliar leaves; *B*, nodes of cataphylls. The sheath enclosing the foliar leaves of the new corm is shown at the center. $\times 1.5$. FIG. 3. Old and new corms at time of flowering: *A*, contractile root drawing offset from parent corm, the epidermis acting as a sheath through which the cormlet is being lowered; *B*, absorbing roots of the old corm. The outer sheath is that of the old corm. The sheath of the new corm is shown enveloping the leaves. $\times \frac{1}{4}$. FIG. 4. Semi-diagrammatic section through mature flowering corm and old corm: *A*, flower stalk; *B*, vegetative bud of new corm; *C*, new corm; *D*, old corm with absorbing roots; *E*, sheath of new corm (sheath of old one has been removed); *F*, cataphyll; *G*, outer foliar leaf. $\times 2.6$.

which connects the corm with the old corm of the preceding season, to approximately the surface of the soil. It is the older corm which possesses the absorbing roots at this time. The flowering corm is fairly packed with starch grains. Raphide cells are present throughout but are more numerous in the periphery and near the base of the corm. The crystals are imbedded in a mucilaginous mass within the cell.

The outer sheath becomes very tough and fibrous towards the end of the flowering period because of the lignification of tissues connected with the vascular bundles (text fig. 3). The flower stalk and leaves wither away and the epidermal cells of the cataphylls become heavily suberized, preventing the drying out of the growing point beneath them. Later these cataphylls become dry and scaly. An abscission layer appears across the "neck," cutting off the old corm, which by this time is shrunken and practically devoid of starch. In this condition the corm passes through the very dry summer which is characteristic of this region.

With the coming of the fall rains, in September or October, the corm resumes activity. Numerous adventitious absorbing roots develop from the central axis. A new corm with two foliar leaves starts its development in the terminal cavity of the old corm and a flower umbel appears in the axil of the second leaf. The vegetative bud grows rapidly so that before cold weather sets in the new corm is well developed (text fig. 1).

Axillary buds arise at the nodes of the old corm, forming offsets which develop a protecting sheath, a single leaf, and the rudiments of a root (text fig. 1) before winter brings on a second period of dormancy. These offsets may appear at either of the two nodes which bore foliar leaves (text fig. 2) or at either of the two cataphyllary nodes (text fig. 1). The number borne on each corm varies from one to ten though two or three are usually found. They may appear singly at separate nodes or several in the same leaf axil.

In the spring the growth of the small corm imbedded in the top of the old corm continues. The small vegetative bud of the new corm becomes sunken because of the large amount of parenchyma tissue formed around the apex by the procambium mantle. Since the leaves have little or no surface above the soil at this time all of the growth is at the expense of the older corm. It was found that the starch is digested from the lower portions of the corm first, then, as the season progresses, the food reserve is withdrawn from the periphery. By the time the abscission layer is formed there are very few starch grains remaining except around the central axis and upper portion of the older corm. The cells of the new corm are practically filled.

DEVELOPMENT OF THE OFFSETS

Each offset produces only a single contractile root. This is at first enclosed in what might be termed a "coleorhiza" (text fig. 18) which soon becomes ruptured as the root elongates. The root grows out over the side

of the corm and downward so that, upon contraction, the cormlet is drawn downward and away from the parent corm. Instances were found where the offset had been moved approximately three inches by the single contractile root. An inch and a half or two inches is the rule. In the fall of that season the offset produces a crop of adventitious absorbing roots but never another contractile root. This means that the large corms found deep in the soil have reached their present position by means of several generations of offsets.

From the number of old corms found beneath corms flowering for the first time it is judged that an offset vegetates for four to five years before producing a flower stalk. It may produce offsets in the meantime but this occurs rather seldom. To determine whether or not a corm was flowering for the first time the old corm beneath it was examined for the mass of vascular traces which run from the central axis to the flower stalk (text fig. 4). During the period of vegetation the corms produced bear only a single foliar leaf. When a corm is developed that is of sufficient size to store the requisite food material for flowering, two foliar leaves appear along with the flower stalk (text fig. 1).

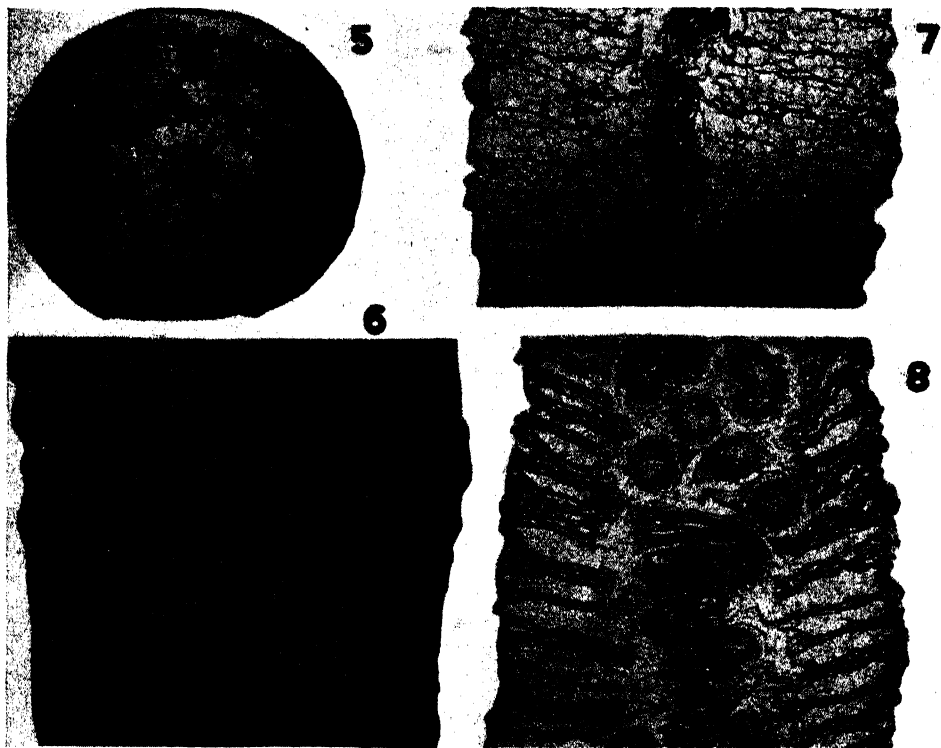
THE CONTRACTILE ROOTS

In *B. lactea* the contractile roots appear only on an offset and never on a parent corm. They vary from 4 to 15 cm. in length, most of them being 6-10 cm. long, and from 2 to 4 mm. in diameter. Contraction takes place in the upper parts of the root while it is still growing at the tip so that its entire length cannot be used in measuring the degree of contraction. When a single part of the root was taken, however, it was found that there is a shortening in length of at least 75 percent. Lateral roots originate in the pericycle but seldom reach the surface of the root under natural conditions. This is due to the changes undergone by the cortical cells during contraction that cause the death of the cortex before the lateral roots are fully developed.

The absorbing roots possess a triarch stele with a large, central metaxylem cell. The ratio of the diameter of this central cylinder to that of the root as a whole is about 1-4. The ratio in the contractile roots is 1-8, the stele itself being somewhat larger.

The stele of the contractile roots is apparently pentarch although in many of the larger roots as many as eight groups of xylem are found. These are quite regular in their distribution (text fig. 17) so that there seems to be no possibility of this increase in the number of xylem strands being the cause of either the contraction of the root or the distortion of the stele. The same figure shows that the parenchyma cells of the central cylinder are regular as seen in cross section and not in the least distorted. It seems probable, therefore, that some tissue other than those found in the stele is responsible for the contraction of the root. This central strand is straight

at first but in a region of maximum contraction is twisted back and forth upon itself many times, with smaller waves showing in the larger loops. This is not in the form of a true spiral but is very irregular, the undulations extending in all directions.



TEXT FIG. 5. Transverse section of contractile root through region where collapse of cells is well under way, showing the vascular stele somewhat distorted, the unequal horizontal distribution of the collapsed tissue, and the sloughed epidermis. $\times 14$. FIG. 6. Longitudinal section of contractile root showing the result of a regular distribution of the collapsed tissue, the stele making an abrupt bend. $\times 14$. FIG. 7. Longitudinal section of root showing an unequal distribution of the collapsed tissue, resulting in the gradual distortion of the stele. $\times 25$. FIG. 8. Longitudinal section of root through the region of maximum contraction showing the further collapse of the cortical cells and the distortion of the stele. $\times 14$.

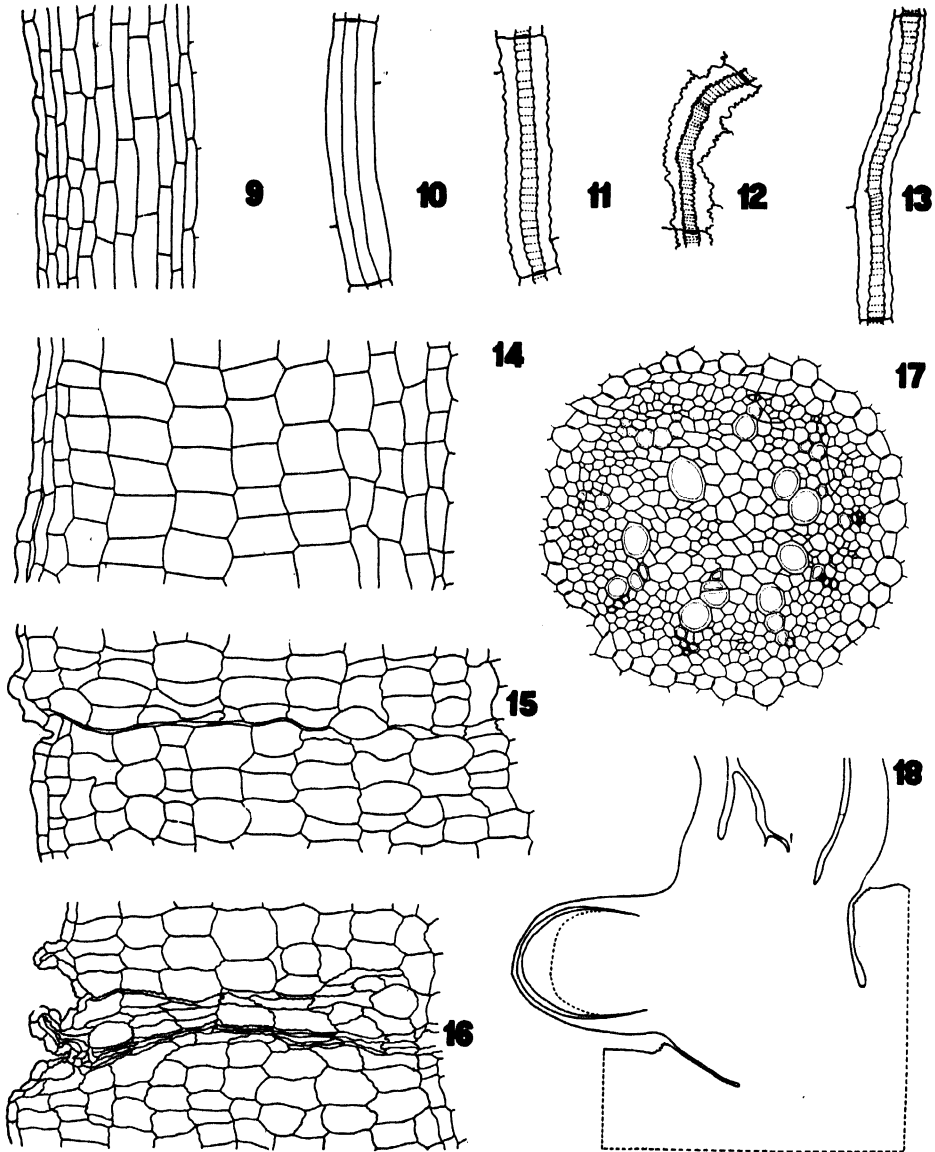
There are very definite changes in the parenchyma cells of the cortex during contraction of the root. Back of the growing point the cells elongate rapidly as they do in normal roots (text fig. 9). In the region of differentiation and immediately above it the cortical cells shorten in length and increase considerably in their radial and tangential measurements (text fig. 14). A layer or two of cells just underneath the epidermis do not change as much as the cortical cells but do shorten more than the epidermis itself. This accounts for the epidermis becoming somewhat loosened at this time. Also a layer or two of cells adjacent to the endodermis are not changed to

the same extent as the cortical cells. The walls of the endodermal cells become undulated, especially the radial walls reinforced with the Casparian strips (text fig. 11). This results in a slight shortening of the cell. This shortening is undoubtedly due to the pressure created by the decrease in length of the cortical cells. The force would also affect the central strand so that at this stage it would be under a tension on all sides, acting along lines parallel to the stele as well as at right angles to it.

The radial and tangential increase continues, the cells next to the epidermis and endodermis taking a greater part in the process than before, until the cortical parenchyma cells are greatly flattened. The epidermis is completely loosened from the root, remaining as a sheath, while the exodermis becomes slightly suberized and serves as protective tissue. As the root shortens the cormlet is either drawn down through the sheathing epidermis (text fig. 3) or the epidermis is found folded and packed beneath the cormlet.

The process so far has been quite regularly distributed throughout the cortex and the vascular stele remains straight. The tension on the strand by this time is comparatively great. Then there appears to be a sudden collapse of a layer of cells extending from the exodermis to the endodermis (text fig. 15). It is more or less assumed that the collapse starts at the outside and progresses toward the center although this could not be proven absolutely because of the unequal horizontal distribution of the collapsed tissue (text fig. 5). The endodermal cells, as well as some of the outer parenchyma cells of the stele, become even more undulated. These undulations are not confined to the radial walls but appear in all the walls of the cells (text figs. 11, 12, 13). The collapse of cells continues, involving more and more of the turgid cells on either side of them. At this time the remaining turgid cortical cells round off again, increasing somewhat in length and decreasing in their radial and tangential measurements (text fig. 16). Thus, from this stage on, as the root contracts, the diameter of the root as a whole decreases.

If the layers of collapsed cells are fairly regular, that is, if the groups on either side of the stele are more or less opposite each other as seen in a longitudinal section, there will be a comparatively great strain placed on the stele but the force will be about equal on all sides. Sooner or later, however, a change occurs which allows a sudden release of this strain at some point and there the stele makes an abrupt bend (text fig. 6). From this point on to the base of the root the central cylinder is bent and twisted. In most cases the distortion starts more gradually than this. The layers of collapsed cells are opposite to the turgid cells on the two sides of the stele. The collapsed cells do not have the pulling power of the turgid cells, and, as a result, the stele is pulled away from the weakened layer (text fig. 7). In practically all sections the turgid cells appear attached to the central cylinder while the collapsed cells are torn loose, either at the endodermis or at one



FIGURES 9-16 are camera lucida drawings of longitudinal sections of the contractile roots showing changes in the cortical and endodermal cells during contraction.

TEXT FIG. 9. The cortex in region of elongation. $\times 42$. FIG. 10. An endodermal cell in region of elongation showing the radial wall with its Casparian strip. $\times 180$. FIG. 11. Endodermal cell in region of shortening of the cortical cells (see fig. 14). $\times 180$. FIG. 12. Endodermal cell on concave side of a curve of the stele. $\times 180$. FIG. 13. Endodermal cell on convex side of a curve of the stele. $\times 180$. FIG. 14. The cortex in region of shortening and broadening of the cells. The epidermis is becoming loosened. $\times 42$. FIG. 15. The cortex showing further shortening of the cells and the start of the collapse of tissue. The epidermis has been sloughed and is not shown. The exodermis has become thickened. $\times 42$. FIG. 16. The cortex, showing the widened band of collapsed tissue and the rounding off of the remaining turgid cells. Epidermis omitted. $\times 42$. FIG. 17. Transverse section of stele with the attached endodermis, taken from the region of greatest distortion. $\times 90$. FIG. 18. Semi-diagrammatic section of an offset still

or two cells outside of it. Any illustration of the distribution of these collapsed cells represents only two planes. It is necessary to visualize this collapse as occurring in three planes. No doubt all of the collapsed tissue is continuous from where it first appears to the top of the root. All of the smaller patches and layers are in contact at some point. The same is true of the turgid cells. This is not in the form of a spiral arrangement but is very irregularly distributed (text fig. 5).

The collapse of the cells continues until there are only one to three layers of turgid cells remaining between wide bands of collapsed cells. The stele is then at its greatest distortion (text fig. 8). The endodermal cells always remain attached to the stele. On the concave sides of the curves of the strand they are much shortened and distorted (text fig. 12). On the convex sides they are drawn out longer than their original length though some undulations remain in the Casparian strips (text fig. 13).

The thickenings of the tracheal tubes are closer together on the concave sides of the stele than in the normal portions. Approximately fifty percent more bands are found within a given distance than are found in those portions where the central cylinder is straight. Also they are further apart on the convex sides of the curves than they are normally. This is what one would expect if the stele were being passively bent and distorted, and seems to indicate that the process is purely mechanical and that there is no such absorption of material from the walls of the tubes as is postulated by Gravis (6).

Finally all of the cells of the cortex collapse and the root, which until now has been rigid, becomes flaccid and the stele straightens out somewhat when removed from the soil.

According to Sachs (19) there are three things which may create a tension within an organ, the turgor of the cells, the swelling and contraction of the cell walls themselves when they imbibe or lose water, and the changes in volume and form caused by the growth of the cells. Since the walls of the cortical parenchyma cells of these contractile roots are very thin the possibility of their taking an active part in the contraction through the imbibition or loss of water may be disregarded. It is nearer the truth to assume the combination of growth and turgor of the cells to be the direct cause of the contraction of the root and the distortion of the stele in *B. lactea*.

After the cells have elongated they undergo a change in the direction of growth, possibly by the method suggested by Gravis (6). This growth creates a tension on the stele, pulling in all directions. The turgor of the cells aids in maintaining this stress. Then, as a collapse of cells occurs on one side of the strand, the turgid cells on the opposite side will tend to pull the stele in that direction (text fig. 7). When very many of the cells have collapsed the remaining turgid cells are released from some of their external pressure. The turgor of each cell then causes it to round off as

previously described. The initial factors that cause the collapse of some of the cortical cells and not of others could not be determined.

The functions of contractile roots have been variously interpreted. According to Geiger (5) the contractile roots of *Gladiolus* anchor the plant and draw the new corm deeper into the soil, store water, and contribute to the raw material already present in the growing shoot. Rimbach (15) considers that the principal function of the contractile root of *Lilium martagon* is to lower the newly developed bulb into the soil.

Since an offset of *B. lactea* does not produce nutritive roots the spring it originates, all the water and minerals used by it after it has been separated from the parent corm must be absorbed by its single contractile root. There is no indication of reserve food material of any kind in the root, so it does not serve as a food reservoir. The corms growing near the water as a rule produce more offsets than those in drier habitats. Also the roots live only a comparatively short time, three or four weeks, and during this period there is plenty of moisture available. Thus they could hardly be considered as water-storage organs.

The only functions performed by the contractile roots in this case, then, are absorption and the anchoring and lowering of the offsets into the soil. This is more or less corroborated by the fact that after a corm has reached a certain depth, ten to sixteen inches in loose soil, no more contractile roots are formed, even if offsets are produced. Offsets occur very rarely on these deep-seated corms. The corms in rocky soil never reach this depth and if plenty of moisture is available numerous offsets are produced.

SUMMARY

1. The seasonal development of the corms of *B. lactea* and the process of root contraction as it occurs in the roots of the offsets are described.

2. Each year a new corm develops from the terminal bud of the old corm.

3. Offsets are produced by buds in the axils of either the foliar leaves or cataphylls. These offsets vegetate for four to five years before producing flowers.

4. Contractile roots are produced only on offsets and never on parent corms.

5. The cause of the contraction is the combination of growth and turgor of the cortical cells. During the process there is an actual shortening and broadening of these cells, followed by a complete collapse.

6. The stele is greatly distorted by the irregularity of the distribution of the first layers of collapsed tissue, the remaining turgid cells exerting the requisite force. The cause of this irregular collapse could not be determined.

7. The functions of the contractile roots of *B. lactea* are the absorption of raw materials and the lowering of the offset into the soil.

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A PRELIMINARY DESCRIPTIVE STUDY OF A PARASITIC MONAD IN CELLS OF AMERICAN CHARACEAE

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INTRODUCTION

In 1885 Zopf observed and described an organism parasitic in cultures of *Nitella* which had been growing for several months in the greenhouses in Berlin. Infected leaves, nodes, and internodes which in the beginning had been deep green turned brown and golden-brown in color and finally became colorless and died. Later, Zopf also discovered the disease in species of *Nitella* and *Chara* growing in nature. On examining such plants microscopically he found their cells to be filled with an active heliozoic organism to which he gave the name *Diplophysalis stagnalis*. Its life history consists of zoöspores, amoebae, zoösporangia and resting spores. The biciliated zoöspores, according to Zopf, bore their way into the host cell where they shortly become amoeboid, forming numerous fine, extended pseudopods as in *Actinophrys*. The amoebae grow to a large size, become invested with a wall, and finally undergo cleavage into zoöspores. Resting spores or cysts are similarly formed later inside of the primary wall, thus resulting in a double-walled cyst. Its life history is quite similar to that of *Pseudospora nitellarum* (Cienkowski) or *Diplophysalis nitellarum* (Zopf, 1885) described fragmentarily by Cienkowski in 1865 from cells of *Nitella*, with the exception that the inner wall of the resting spore or cyst is highly sculptured and often bears resemblance to a many-pointed star.

Organisms of this nature were for a long time confused with the reproductive organs of the algae. Meyen (1839), Pringsheim (1852), and others believed that they were developmental stages of spores of *Spirogyra*, *Oedogonium* and the Bacillariaceae. Pringsheim designated them as pseudogonidia in 1852, but shortly thereafter (1858) Cienkowski discovered their true nature and proved them to be parasites and totally unrelated to the algae in which they occur. He established the family Monadineae with five genera, *Monas*, *Colpodella*, *Pseudospora*, *Vampyrella*, and *Nuclearia* to include these forms. Cienkowski early noted that they were of two types. Some formed typical zoöspores to which he gave the name Monadineae Zoösporeae, while in others this stage was lacking and they formed at first only *Actinophrys*-like amoebae. These he named Monadineae Tetraplastae.

Our knowledge of these organisms was further extended by the excellent studies of Zopf (1885) who monographed the group as a subordinate class of the Myxomycetes, divided it into four families, added several new genera,

and included the parasitic slime moulds, *Plasmodiophora* and *Tetramyxa*. Zopf likewise recognized two large divisions, Monadineae Azoösporeae and Monadineae Zoösporeae. The genus *Diplophysalis* was established to include the zoösporic forms whose resting spores or cysts have two separate and distinct walls, and *Pseudospora Nitellarum* and *P. Volvocis* thus became *Diplophysalis Nitellarum* and *D. Volvocis*, respectively.

Since the time of Cienkowski and Zopf the Monadineae have been regarded more strictly as Protozoa. Kent (1882) included *D. Nitellarum* in the family Monadidae Ehrenberg of the Flagellata-pantostomata and changed its name to *Monas Nitellarum*, while *D. Volvocis* was placed in the family Pseudosporeae and became again *Pseudospora volvocis* of Cienkowski. In 1887 Bütschli placed the genus *Pseudospora* in the Isomastigoda division of the flagellata. In 1891 Lankester established the class Proteomyxa of the Protozoa to include forms which are difficult to place under the Rhizopoda or Flagellata and which produce simple cysts instead of more complex fructifications like the Myxomycetes. He includes in this class most of the genera of Cienkowski and Zopf, but merely mentions (1909) that *Diplophysalis* seems to be related to *Pseudospora*.

The class Proteomyxa of Lankester has subsequently become well established in the literature and bids fair eventually to displace Cienkowski's and Zopf's Monadineae. Delage and Herouard (1896) make Proteomyxa a sub-class of the Rhizopoda, but still retain the divisions, Monadineae-Azoösporeae and Monadineae-Zoösporeae. In the latter they include *Diplophysalis* and all other but two of Zopf's genera. Hartog (1906, 1909, 1922) retains the class Proteomyxa but regards it as a store room for rhizopod- and flagellate-like organisms which are difficult to classify. Doflein (1909), Doflein and Reichenow (1928), and Calkins (1926) likewise accept Lakester's Proteomyxa but limit it to a few genera.

In the course of my cytological studies on the Characeae numerous greenhouse cultures of *Nitella* and *Chara* have become infected with *Diplophysalis stagnalis*, and I have thus been able to follow the life history and pathogenicity of this organism over a period of years. So far I have not found any additional report on *D. stagnalis* in the literature since the time of Zopf. In view of the fact that this seems to be the first note of its occurrence in cells of American Characeae and that the processes of cleavage and zoöspore and resting spore formation appear to be so unusual, I shall report briefly on its distribution, pathogenicity, and life history, as I have observed it. The data here presented relate only to living material. A more detailed cytological study is now being made on fixed and stained preparations and will be published later.

SYMPTOMS OF THE DISEASE

The macroscopic appearance of this disease is quite distinctive and easily recognizable. *Diplophysalis stagnalis* is a virulent parasite and .

attacks all parts of the plant including the rhizoids. Infected internodes, nodes, "leaves" and stipules soon turn yellowish-green, yellowish-brown, and golden brown in color, so that it is easy to detect the inception and progress of the disease in cultures of *Nitella* and *Chara*. The change in color is due to plastid degeneration. Once a cell has been infected it soon becomes filled with the large amoebae of this organism which engulf degenerating plastids and starch grains lying in the primordial utricle. These plastids are digested, soon degenerate further in color within the amoebae, and turn brown. When the disease has reached a late stage the remaining plastids which have not been engulfed may also degenerate and accentuate the brown appearance of the cells. The host cells later become greyish brown and finally lose their color altogether. Over a period of several months all of the plants in a culture may be attacked to the extent that only a few "leaves" and tips remain alive. On the other hand, I have observed rapidly growing infected cultures which eventually recovered from the attack and continued to grow normally again.

LIFE HISTORY OF THE CAUSAL ORGANISM

Diplophysalis stagnalis, according to Zopf, has four distinct stages in its life history: zoöspores, amoebae, sporocysts, and resting cysts. It begins as a flagellated organism, and therefore belongs in the zoösporic division of the Monadineae. In this respect it is unlike *Vampyrella*, *Spirophora*, *Leptorhrys*, *Bursulla*, and other members of the group. In Plates LVI and LVII I have shown the developmental stages of the zoöspores, amoebae, and zoösporangia.

As Zopf described, the zoöspores escape at several places from the sporangium. They are generally oval, oblong, or decidedly pear-shaped. They may become quite active or remain sluggish in their movements immediately after escaping. On several occasions I have observed that they remained quiescent for a few moments before swimming off. Figures 1 and 2 show the zoöspores shortly after escaping. I have not yet been able to determine from my material whether they are always bi-ciliated. Occasionally some may be seen which appear to be unflagellated (fig. 2). Moreover, some appear to have their flagella attached anteriorly and posteriorly, but one cannot be altogether certain about this point. The cell wall of the host is at times quite opaque, and the presence of disintegrating plastids and numerous starch grains often renders observation difficult and uncertain in living material. Zopf figures the zoöspores as both uni- and bi-ciliated with anterior and posterior attachment. For *Pseudospora volvocis* or *D. volvocis*, Cienkowski (1865) figures both uni- and bi-flagellated zoöspores, while Robertson (1905) and Roskin (1927) figure only flagellated forms with both flagella at the posterior end. Robertson claims that in swimming one flagellum lashes out in front. If this is true also in *D. stagnalis*, it may account for the appearance of Zopf's and my

own figures. I shall leave this question together with the problems of division and so-called fusion (Robertson) of the zoöspores for a future study.

As Zopf, Robertson, and Roskin have shown for the forms with which they worked the zoöspores may become amoeboid while the cilia still remain attached. This is well illustrated in figures 3 and 3a. The zoöspores do not generally engulf food, but once the amoeboid form has been assumed, active feeding begins. Contractile vacuoles which gradually enlarge and then quickly disappear may often be seen at this stage. As the zoöspore assumes the amoeboid stage it becomes quite irregular in shape with comparatively few blunt and irregularly located pseudopods. Later on it assumes what may for convenience be called a rounded or radial stage, figures 4, 5, and 6, with numerous fine, more or less radially extended pseudopods from all parts of the body. These pseudopods may often be twice as long as the diameter of the central portion of the amoeba and run out to an almost invisible fineness. It is in this radial stage that the organisms bear such close resemblance to the Heliozoa.

At this stage (fig. 4) one can often detect a differentiation into ectoplasm and endoplasm. At times, however, the differentiation is not so marked. Figure 5 shows a semi-radial stage in which are a few granules and starch grains. The longitudinally extended and bi-polar shape is perhaps the most characteristic of the organism while actively feeding. As it feeds it creeps along slowly in the primordial utricle of the host cells engulfing degenerating plastids and starch grains. The plastids turn yellowish and golden-brown in color as they are digested, filling the endoplasm with innumerable granules and giving the amoebae and the infected host cell their characteristic appearance. Figures 6 and 7 show radial stages of large amoebae which are almost filled with starch grains, granules and large globules of various nature. They may be brilliant brown in color at this stage as a result of the partially digested and degenerated plastids. It is difficult to show in only black and white figures the characteristic appearances of such stages. Zopf's beautiful colored figures illustrate these stages more adequately.

When the host cells are rich in starch grains the large amoebae may become literally gorged, so that they look like balls of starch grains, disintegrating plastids, and various other granules held together by a relatively thin layer of protoplasm. As they become large and filled with food, the fine ray-like pseudopods appear to be retracted and become shorter and more blunt (figs. 7 and 8). The organism then becomes less like a Heliozoan and more like a plasmodium in appearance. Figure 9 shows a large amoeba in which the sharp pointed pseudopods are entirely absent. Such amoebae are very slow and sluggish in their movements.

I have interpreted these changes as an indication of maturity and a preparation to form the sporangium or sporocyst. Finally the amoebae

round up completely, apparently lose their motion, and become almost spherical in shape as is shown in figure 10. Frequently at this stage one may note very active Brownian movement. In fact, it may become so active and violent that the starch grains and various granules are made to dance about. I doubt if more active Brownian movement is to be observed in any other cells in either the plant or animal kingdom. Such active movement may be a sign of degeneration and death, since I have so far failed to observe amoebae of this nature undergo cleavage into zoöspores.

FORMATION OF THE SPOROCYST OR ZOÖSPORANGIUM

The large amoebae which have rounded up eventually become invested with a thin wall or membrane as is shown in figure 11. This may be taken as the first step in the differentiation of the zoösporangium. The next stage, as Zopf has shown, is the gradual aggregation of the apparently excess unassimilated starch grains and granules. This material may eventually lie somewhat loosely or densely aggregated in the center of the body or slightly off towards the periphery. These grains and granules may be excretory substances and also an excess of food material which have been engulfed by the organism in its active feeding stage. If this is the case we have here a very unusual condition in which an organism eats or engulfs food far in excess of what it assimilates. That these are normal unaltered starch grains can be readily shown by testing with iodine and staining with Flemming's triple stain. Furthermore, as will be shown below, masses of unaltered starch grains may persist throughout the entire process of zoöspore formation and finally be liberated into the host cell by the breaking down of the empty sporocyst wall. The manner in which the excess material moves toward the center or any particular region is difficult to understand. The effect of the Brownian movement on these particles is very suggestive, but it is difficult to conceive how such movement becomes oriented to a definite purpose. Moreover, Brownian movement, as I have noted before, appears to be a sign of degeneration rather than a stage in sporangium development.

The significance of this centripetal aggregation in relation to the process of cleavage is apparent. If the excessive food material remained indiscriminately scattered throughout the sporangium it would obviously interfere with the process of cleavage. The protoplasm which is to be propagated as zoöspores is hereby freed of all excess materials as in many of the Sporozoa and slime moulds. In both of these groups, however, the process is quite different. In the slime moulds numerous vacuoles are found into which the waste substances flow in soluble form rather than as grains and granules. Zopf does not describe vacuoles in encysted amoebae of *Diplophysalis stagnalis*, nor have I so far been able to observe such organs in which this material collects. This phenomenon of aggregation and elimination has been variously interpreted as a reorganization, rejuvenation, or de-differentiation of the protoplasm in preparation for reproduction.

Figure 12 shows an incipient sporocyst in which the waste material is loosely aggregated in the center. Shortly after this stage cleavage begins as is shown in figures 13 and 14. According to Zopf it is simultaneous throughout the entire body, but I have not yet been able to determine whether or not this is generally true. Nonetheless, cleavage or zoöspore formation involves most of the protoplasmic mass except the aggregated excess material. At times the zoöspores are cut out leaving a thin scalloped cortical layer of protoplasm on the inner periphery of the wall, figures 13 to 16. Such cases are very suggestive in appearance of gamete production in *Ophryocystis mesnili* (Leger, 1907) where the gametes are cut out leaving a cortical layer of protoplasm which forms the so-called "brood chamber." However, in a large number of the fixed and stained preparations of this stage which I have observed, the cortical layer of protoplasm has been absent, as is shown in figure 17.

This method of spore formation in living material thus appears to be really unique. It is quite unlike the process in sporangia of slime moulds and higher fungi (Harper, 1899, 1905, 1914; Swingle, 1903; and Schwartz, 1922) and in many animal eggs where cleavage furrows are quite conspicuous. It is to a certain degree suggestive of free cell formation in *Ephedra* (Strasburger, 1880) and the ascus (Harper, 1897) where a certain amount of protoplasmic residue is left behind. It remains for further study, however, to determine whether or not kinoplasmic activity is here involved as in free cell formation.

There is a wide gap in my observations between figures 12 and 13. Because of the opacity of the host cell wall it is difficult to observe correctly the inception and progress of cleavage in living material. In figure 13 the zoöspores are sharply delimited, and the appearance of the cortical layer of protoplasm suggests their manner of origin. The excess material in the center is densely aggregated. Figure 14 shows a later stage in which a number of the zoöspores have developed cilia. Hartog's figure 29 of *Pseudospora lindstedtii* is very suggestive in appearance of this figure. After the cilia have been fully formed, the zoöspores become very active within the sporocyst, and the continued beating of the cilia and movement of the zoöspores quite often push the ball of excess material to one side and reduce the scalloped border of protoplasm to a thin layer. This is well illustrated in figure 15 in which a number of zoöspores are also shown escaping. The zoöspores often come to lie on one side of the mass of excess food material and generally make their escape through this portion of the membrane.

According to Zopf they may escape in several places from the sporocyst or zoösporangium, boring their way through as well as dissolving a hole in the wall. In fixed and stained preparations, however, the sporocyst membrane often appears broken down and torn in numerous places, but this appearance may perhaps be the result of fixation and sectioning. As

is shown in my figure 15 from living material they may often be drawn to a fine point as they pass through the membrane. The motion appears to be that of slow gliding, very similar in appearance to the passage of nuclei through cell walls into adjacent cells. The passage of the zoöspores often requires several moments, and during the process they may undergo extended contortions in shape and pseudopod formation as if straining to get free. Numerous contractile vacuoles may often be seen at this stage. After all of the zoöspores have escaped only the sporocyst remains with a mass of waste material of varying amount at one side or in the center, and often a thin layer of protoplasm on the inner periphery of the wall as is shown in figure 16. Whether or not this layer, when present, is also used up in zoöspore formation I have so far been unable to determine.

FORMATION OF THE RESTING SPORE OR CYST

According to Zopf, the resting spore has two walls and resembles a many-pointed star within a sphere. The preliminary stages in spore formation are similar to those in the development of the zoösporangium. Large amoebae round up and form a wall or membrane in the same manner, while the waste material is similarly aggregated in a mass and becomes displaced somewhat to one side as a preparation for its exclusion. The remainder of the protoplasmic content then appears to separate from this mass, aggregates and forms a rough walled, highly sculptured resting spore with a finely granular content. It is thus formed within the primary membrane, resulting in a double-walled cyst or spore, according to Zopf, which he takes as the outstanding characteristic of the genus *Diplophysalis*.

Zopf (1885) reports that these cysts invariably follow an infection with zoöspores and amoebae, and figures (Pl. III, figs. 24a, 24b, and 25) successive stages in their development. I have likewise found them present wherever amoebae and zoösporangia occur, but I have been unable to trace the successive stages of their development from encysted amoebae such as are shown in Plates LVI and LVII. In *Pseudospora volvocis* or *D. volvocis* Robertson was unable to find resting spores and thereby questions the validity of Zopf's observations. Roskins, on the other hand, observed and figured the resting spores of *D. volvocis* and a new species *D. endorini*. Their development, according to him, is essentially similar to that of *D. stagnalis*. It is also to be noted in this connection that they also resemble very closely the cysts of *Asterocystis*. While the developmental stages figured by Zopf are fairly consecutive the possibility that these structures might be spores of another organism must not be excluded. Although I have been unable to follow their development, I am, nonetheless, inclined at present to favor Zopf's view.

In the process of cyst development the excess starch grains and other material are again apparently left on the outside. It is to be noted again, that in the formation of the resting spore as well as in the cleavage of the

sporangium the organism eliminates waste materials that have been accumulated. In the resting spore this material may lie to one side within the primary wall as is shown in figure 19, or be so abundant as to envelop the entire spore. This is quite likely to be the case when the host cells are rich in starch. In late winter and early spring the excess material may be considerably less. In the cysts shown in figures 21 and 22 practically no excess material is present.

The secondary wall of the resting spore is characteristically marked. As a result the spore has the appearance of a round or oval body whose surface is covered with many more or less blunt projections. Some of them may be quite sharp, but they never run to a point as spines. They may be few in number (figs. 19 and 22) or very numerous (figs. 18, 21, 22). The primary smooth wall may sometimes disintegrate and break away leaving the spore free (figs. 20 and 21), but it generally persists for a long time.

Zopf did not observe or describe germination of the cysts or resting spores. I have also been unable to find convincing stages of germination. Figures 18, 19, and 20 show the appearance of the spore contents as they may frequently be found. In figure 18 the cytoplasm is finely granular with relatively few inclusions, in figure 19 it is filled with globular bodies and granules of various sorts, while in figure 20 a large vacuole occupies the center. Whether or not these are stages preparatory to cleavage into zoöspores has not been determined.

In figure 21 is shown a cyst whose content appears to be segmented into a number of angular and hexagonal portions. This cyst was kept under observation for several days, but no further development occurred. I am thus unable to say whether or not the segments are incipient zoöspores. In old decaying cells of *Chara* and *Nitella* it is not uncommon to find numerous cysts which have apparently germinated. Such a cyst is shown in figure 22. The primary and secondary walls are still intact, and the spore is empty except for a few granules. It is evident from such figures that if the two walls persist the zoöspores must penetrate both to escape to the outside.

DISTRIBUTION

Diplophysalis stagnalis does not appear to be very specific in its host relation or geographical distribution. Zopf found it in *Nitella mucronata*, *N. flexilis*, and *Chara fragilis*, both in cultures and in nature. I have to date observed it in cultures of thirteen species and four genera including *Nitella flexilis*, *N. gracilis*, *N. tenuissima*, *N. glomerulifera*, *N. opaca*, *Chara fragilis*, *C. delicatula*, *C. coronata*, *C. contraria*, *C. zeylanica*, *C. gymnopus*, *Lamprothamnus alopecuroides*, and *Lychnothamnus barbatus*. Whether or not the forms occurring in these various host species may be physiological species remains of course to be determined.

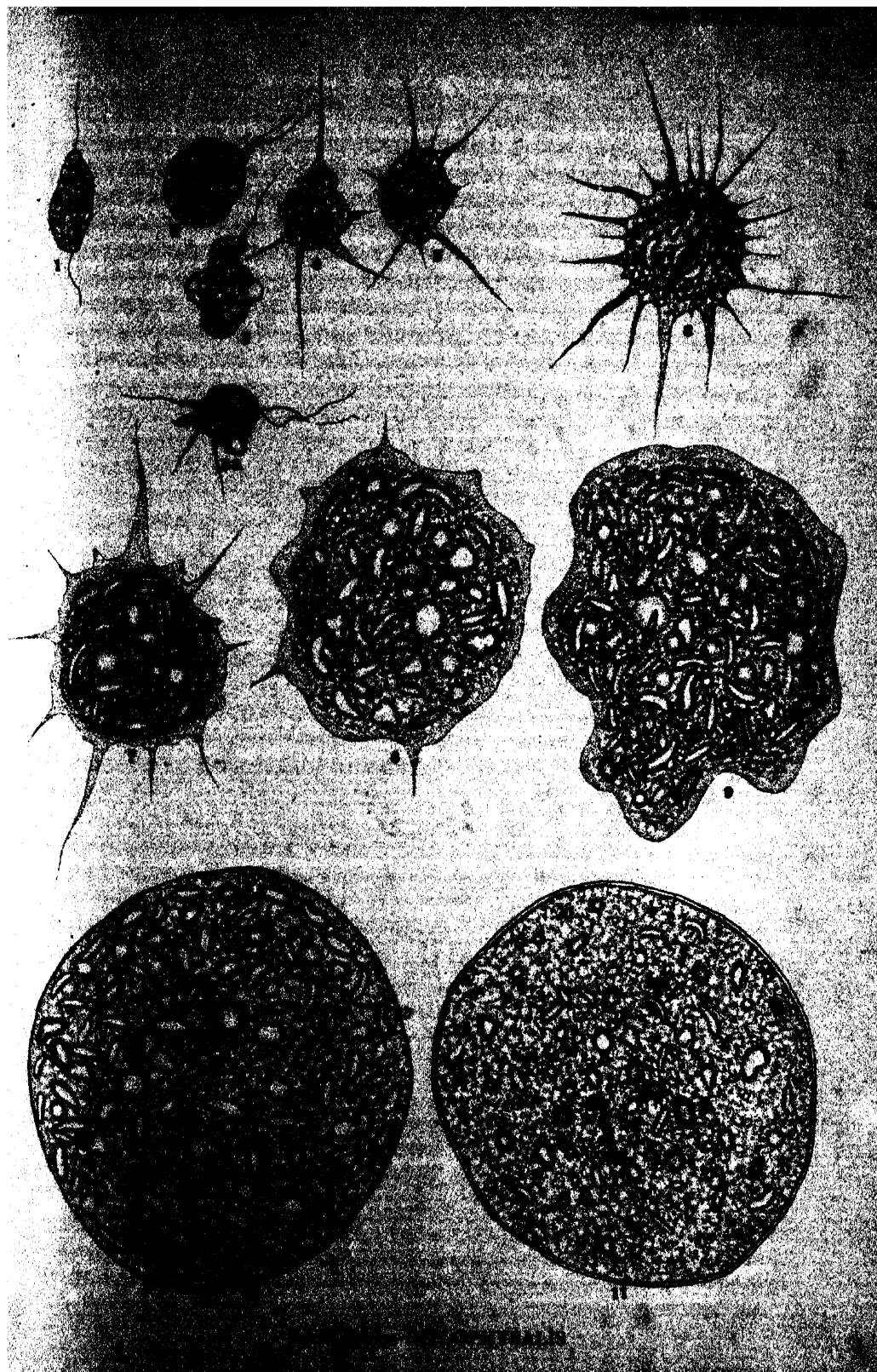
In nature I have found it in *Nitella flexilis* and *Chara delicatula* and *C. coronata* in New York; *Nitella gracilis* and *Chara zeylanica* in Texas,

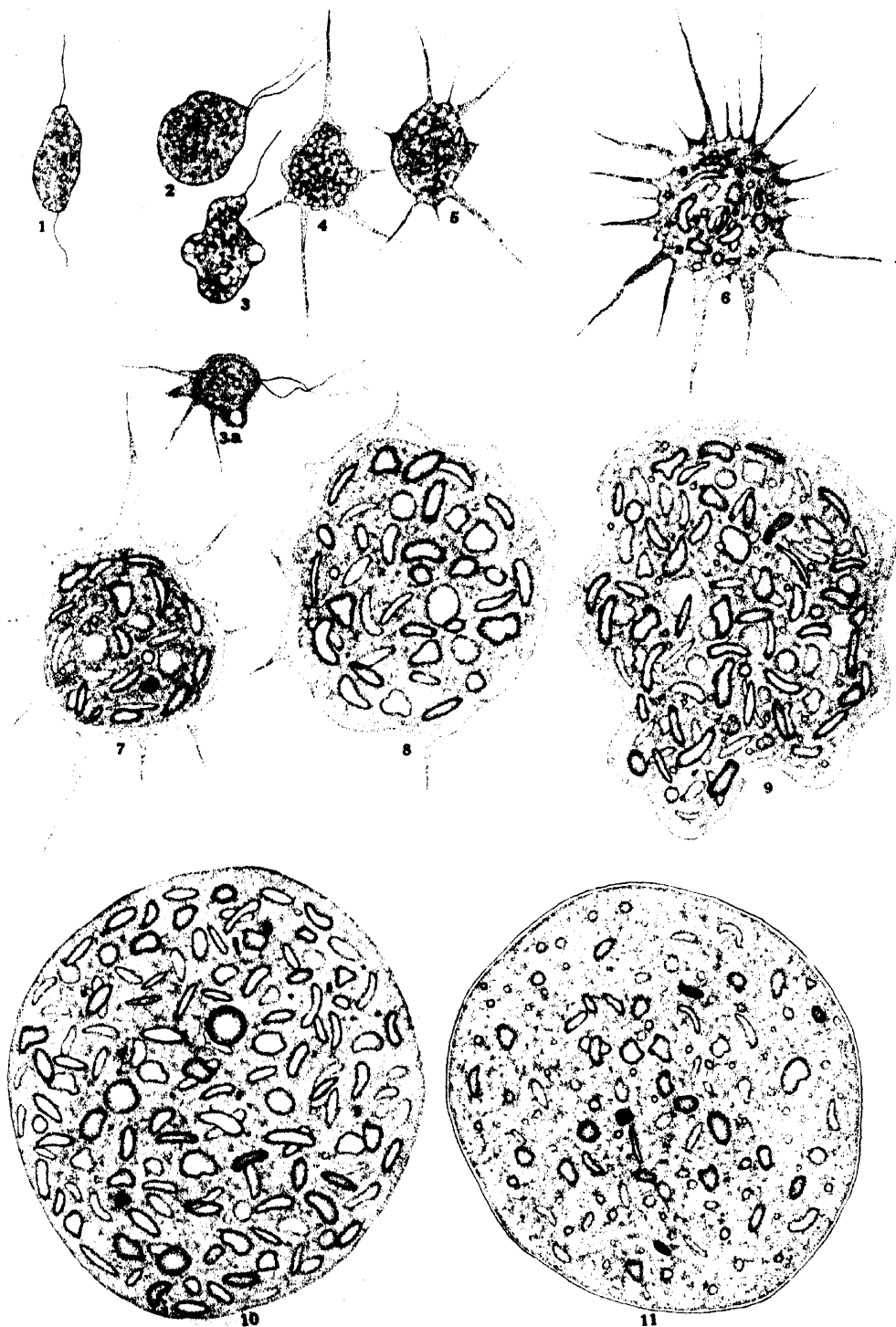
Mexico, British Honduras and Guatemala; *Chara coronata*, *C. zeylanica*, *C. gymnopus*, and *Nitella glomerulifera* in New Jersey; *Chara fragilis* in Wisconsin and Ohio and *Nitella flexilis* in Delaware and Pennsylvania. It appears to spread readily from one Charophyta species to another, but I have so far failed to observe it in other algae growing in the same cultures. The appearance, however, of the resting spores in *Spirogyra* cells as shown by Pringsheim (Pl. 5, fig. 4) is very suggestive of *Diplophysalis stagnalis*. Infected Charophyta cells have been repeatedly placed in *Spirogyra* and *Vaucheria* cultures without results. Nonetheless, other very similar Monadineae, *Pseudospora*, for instance, occur in *Spirogyra*, according to Cienkowski.

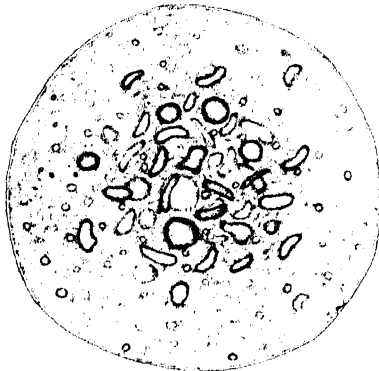
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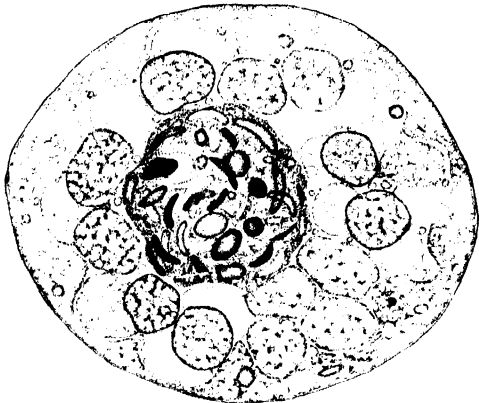
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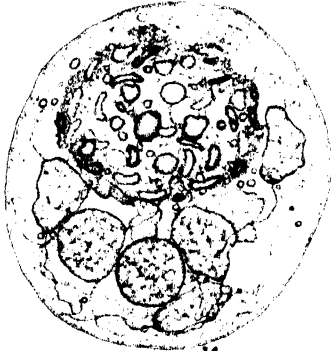




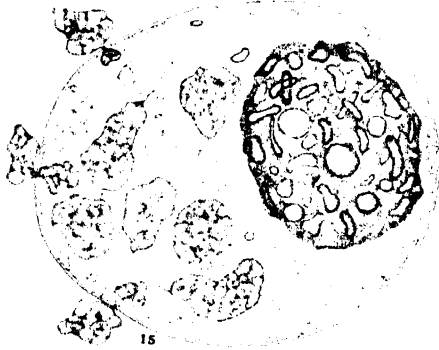
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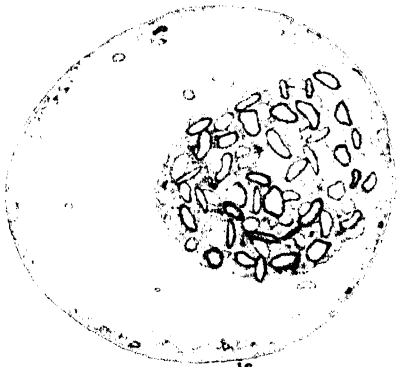
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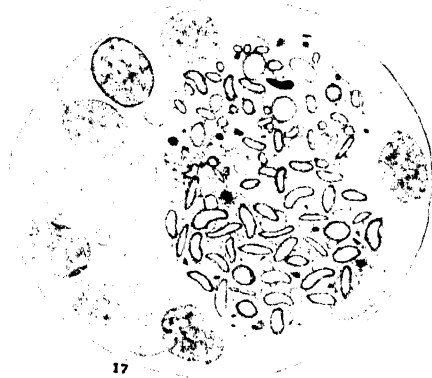
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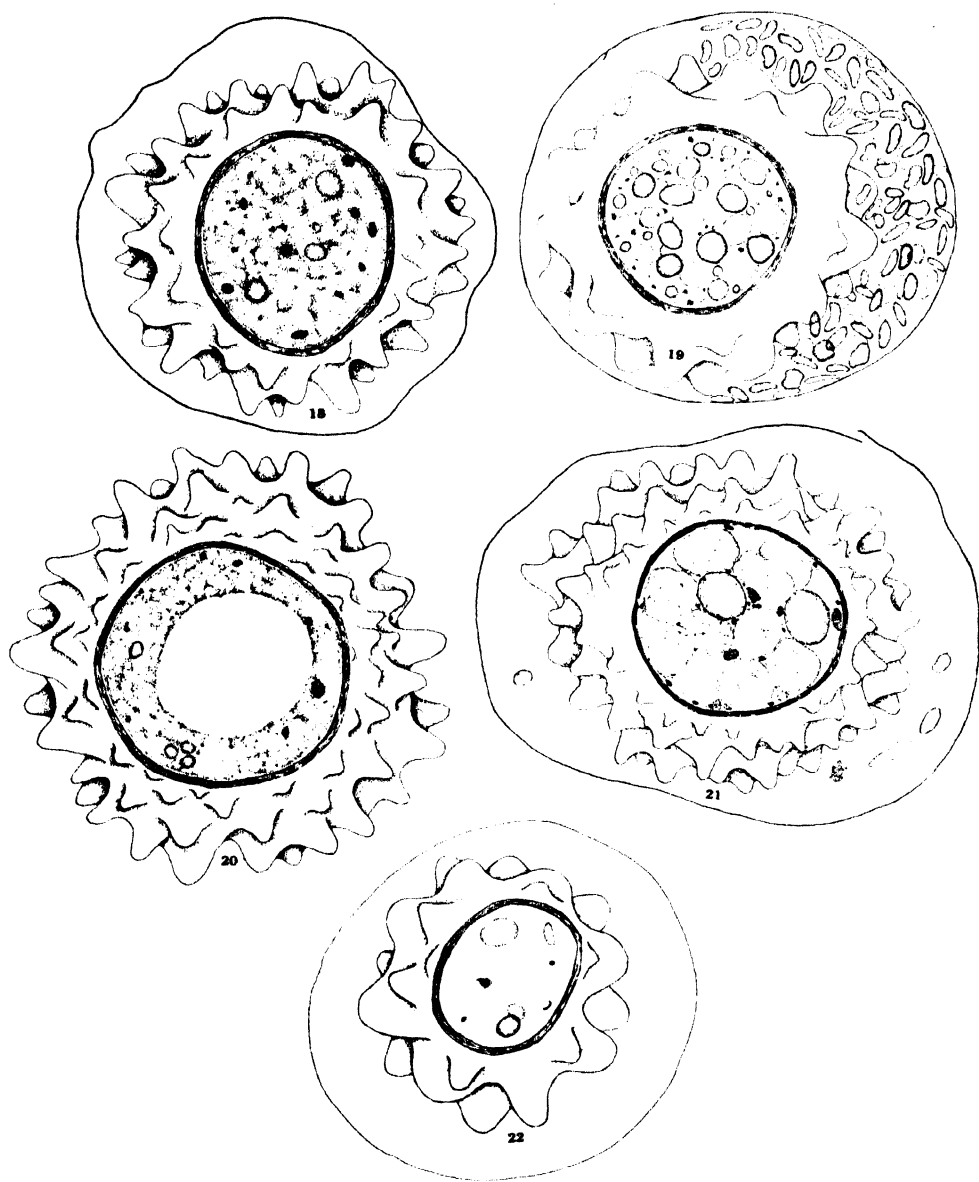
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DESCRIPTIONS OF PLATES

All figures were drawn from living material with the aid of a Spencer camera lucida and a Zeiss 2 mm. Apochromatic objective N.A. 1.30 and compensating ocular No. 6.

PLATE LVI

- FIGS. 1, 2, and 3. Various appearances of motile zoöspores from a cell of *Nitella*.
FIG. 3a. A zoöspore showing pseudopod formation prior to losing its cilia.
FIGS. 4 and 5. Later stages showing the development of extended pseudopods.
FIG. 6. A so-called radial stage with numerous ray-like extensions and engulfed starch grains.
FIG. 7. A later radial stage in which the ectoplasmic layer is visible.
FIG. 8. A large amoeba with relatively few fine pseudopods. The interior is filled with starch grains and granules of various sorts.
FIG. 9. A later stage in which the amoeba is gorged with food. At this stage no sharp pseudopods are present and the organism is very sluggish in its movements.
FIG. 10. A rounded stage preparatory to sporocyst formation.
FIG. 11. An amoeba which has rounded up and become invested with a membrane.

PLATE LVII

- FIG. 12. An incipient sporocyst in which the unassimilated food and excess material has congregated towards the center.
FIGS. 13 and 14. Stages in zoöspore formation.
FIG. 15. A later stage showing the escape of the zoöspores.
FIG. 16. An empty sporocyst with a mass of excess material and a thin layer of cortical protoplasm.
FIG. 17. A similar stage, but one in which no cortical protoplasm is present.

PLATE LVIII

- FIG. 18. A cyst or resting spore within the primary wall. The secondary wall is highly sculptured.
FIG. 19. A similar cyst whose contents is coarsely granular with numerous globules. Between the primary and secondary walls is a mass of excess material which was apparently excluded.
FIG. 20. Another cyst in which the primary wall has disappeared. A large vacuole occupies the center.
FIG. 21. A cyst whose content appears to have undergone segmentation.
FIG. 22. An empty cyst which has apparently germinated.

STUDIES IN THE ANATOMY AND MORPHOLOGY OF THE COMPOSITE FLOWER I. THE COROLLA

MINNA FROTSCHER KOCH

(Received for publication May 1, 1930)

The problem of the venation of the corolla of the Compositae has long attracted the attention of botanists, but as yet a study based on anatomical evidence and explaining the relationship between the ray and disk corollas has apparently not been made. The purpose of the present series of investigations on the composite corolla is to determine its anatomy and comparative morphology. As a result of these investigations it is possible to show the relations between the ray, bilabiate, and disc corollas, and to throw some light on the relationships of the Compositae.

In this first paper, the relation of the ray corolla to the disk corolla is considered, and the gross morphology of the corolla and the floral anatomy of such forms as *Aster* and *Solidago* are discussed.

HISTORICAL

The earliest mention of the venation of the corolla appears in Grew's *Anatomy of Plants* (1682). In speaking of syngenesious flowers, Grew (15) says, "and the Edges of these little Flowers, are frequently Ridged, or as it were, Hem'd like the edge of a Band." No notice either in text or figures, is taken of the disposition of the veins in the corolla.

In 1760, Van Berkhey, in his dissertation on Compositae, published at Leyden, also makes no mention of the veins in the corolla. Schmidel and Schkuhr (2) represent the trunks of the nerves as ending in the sinuses, but they failed to notice the marginal branches. In some of the figures in his plates Schkuhr represents the nerves passing through the axes of the laciniae or lobes (Brown, 2, p. 81).

From 1814 to 1816 three workers, Mirbel, Cassini, and Robert Brown, presented papers explaining the disposition of the veins in the composite corolla. Brown, in his paper read before the Linnean Society of London in 1816, accuses both Mirbel and Cassini of using his information, and establishes his priority. Brown's (2) observations were made when he was classifying the Composites of New Holland, the work being appended to Captain Flinder's *Voyage to Terra Australis*, and are as follows:

It consists in the disposition of its fasciculi of vessels or nerves; these, which at their origin are generally equal in number to the divisions of the corolla, instead of being placed opposite to these divisions, and passing through their axes as in other plants, alternate with them; each of the vessels at the top of the tube dividing into two equal branches, running

parallel to and near the margins of the corresponding laciniae, within whose apices they unite. These, as they exist in the whole class and are in great part of it the only vessels observable, may be called primary. In several genera, however, other vessels occur, alternating with the primary, and occupying the axes of the laciniae; in some cases these secondary vessels being most distinctly visible in the laciniae, and becoming gradually fainter as they descend the tube, might be regarded as recurrent; and originating from the united apices of the primary branches; but in other cases, where they are equally distinct at the base of the tube, this supposition cannot be admitted. A monopetalous corolla not splitting at the base is necessarily connected with this structure, which seems so peculiarly well adapted to the dense inflorescence of Compositae; the vessels of the corolla and stamina being united and so disposed as to be least liable to suffer by pressure (pp. 77-78).

David Don, in 1828 (8), published his account of the ligulate ray in *Zinnia*, and drew definite conclusions as to the nature of this organ.

In 1874, Friedrich Haenlein (16), published his contention that the bilabiate corolla showed a development intermediate between that of the ray corolla and that of the tubular or disk corolla.

Eichler, in 1875 (5), describes briefly the types of venation in the various tribes using the work of Robert Brown as a basis.

Van Tieghem (15) in his *Traite de Botanique* outlines the various kinds of venation found in the Compositae, but does not suggest any relationships.

O. Hoffmann (10) presents the various arrangements of the veins in the corolla and dismisses the discussion as an unsettled question by simply stating that in contrast to other gamopetalous families, the corolla of the Compositae is peculiar.

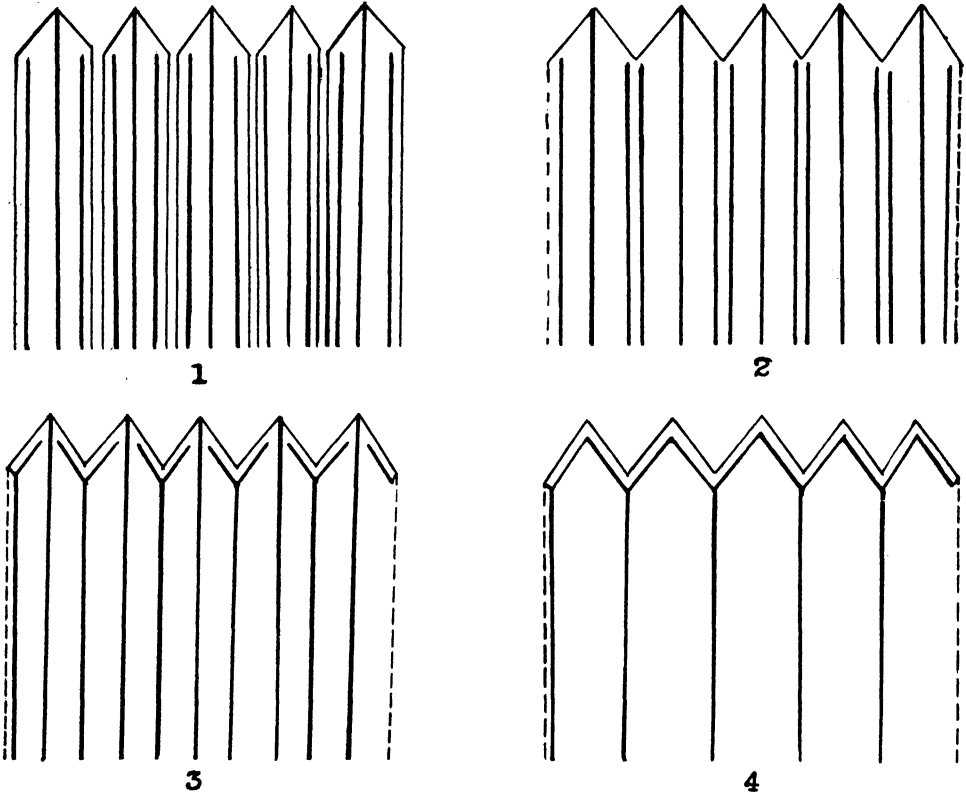
From this date (1897) until 1917 there is apparently no work that pertains definitely to the venation in the corolla. Small (12, 13) recapitulates the work of the previous writers; and especially in the latter paper, the floral anatomy of the three typical forms of florets, tubular, ray, and bilabiate, are considered. It was thought that an examination of the floret of *Tussilago Farfara* L., which Small terms bilabiate, would show an intermediate stage between the ligulate floret, as in the Cichorieae, and the tubular floret, as in the Senecioneae. His results were negative, and he concludes that "the ligulate florets of the Cichorieae have a comparatively constant type of vascular anatomy; that the tubular-disk florets show a slightly greater variability, while the variation in the vascular anatomy of the bilabiate ray florets is so great that they can be distinguished from the first two classes by floral anatomy alone."

METHODS

For the examination of the gross venation in the corolla temporary mounts were made in a weak solution of phloroglucin. A drop of concentrated hydrochloric acid was added to the mount. Flowers used in the examination of the floral anatomy of the floret were killed in 70 percent alcohol and thoroughly dehydrated and cleared in Butyl alcohol (16) before imbedding in soft paraffin. The sections were stained in crystal violet and erythrosin in clove oil.

VENATION OF DISK AND RAY COROLLAS

A gamopetalous corolla is believed to be derived from a polypetalous corolla by the fusion of the petals. A change in the venation of the corolla accompanies this fusion (text fig. 1). Each petal in many polypetalous



TEXT FIG. 1. Diagram of the venation in a polypetalous corolla. The vascular supply to each petal consists of one median bundle and two lateral bundles. FIG. 2. Diagram of the venation in a primitive gamopetalous corolla. FIG. 3. Diagram of the venation in a primitive composite corolla. The lateral bundles of adjacent petals have fused. FIG. 4. Diagram of the venation in a typical composite corolla. The median bundles have been lost and there are five fused lateral bundles which divide at the sinuses and run along the margins of the corresponding lobes.

corollas has three vascular bundles, two of which are lateral bundles, and one a median bundle. Thus a corolla of five petals contains a vascular supply of fifteen bundles. In the fusion of the petals to form the gamopetalous corolla, two lateral bundles of adjacent petals eventually fuse phylogenetically (text figs. 2, 3), and there is then a vascular supply of ten bundles, five fused laterals and five medians. The primitive composite corolla has a supply of ten bundles (Pl. LIX, fig. 6), five of which are fused laterals, equivalent to ten bundles, and five are medians (text fig. 3). The supply in such forms as *Helianthus divaricatus* L. (fig. 3), *Erechtites hieraci-*

folia (L.) Raf., *Arctium Lappa* L., *Anaethra ilicifolia* Don., and others, consists of ten bundles. In the disk corollas of practically all of the Compositae the median bundles have been lost and there are five fused lateral bundles which divide at the sinuses and run along the margins of the corresponding lobes (text fig. 4).

A series of corollas may be found to illustrate the reduction from the most primitive type of venation, fifteen veins (ten laterals, five medians, Pl. LIX, fig. 1) to ten veins (five fused laterals, five medians, fig. 3), to five veins (five fused laterals, fig. 4) and finally to a stage in which the five fused laterals terminate at the sinuses (fig. 5). Illustrations of the steps by which the reduction in the venation of the corolla is brought about is observed in the following forms. *Senecio Fremontii* T. and G. (fig. 2) shows the median bundles extending through only a portion of the corolla and having no connection with the bundle system in the achene. *Chrysanthemum Leucanthemum* L. (fig. 4) shows the fused lateral bundles dividing at the sinuses and running along the margins of the corresponding lobes. *Xanthium orientale* L. (fig. 5) shows the fused laterals terminating at the sinuses.

Five fused lateral bundles dividing at the sinuses and running along the margins of the corresponding lobes is the venation that is dominant for all disk corollas, and for tubular corollas in tribes such as the Eupatorieae and Vernonieae. *Chrysanthemum Leucanthemum* (fig. 4) serves as the type for the venation in the disk corolla.

That this type of venation is not peculiar to the Compositae alone, a review of the families immediately preceding this family in the Engler system of classification will show. The stages that lead to the development of this bundle arrangement are found in *Hamelia patens* Jacq. (fig. 1) and *Pinckneya pubens* Michx., rubiaceous plants which have five-lobed corollas. In these species the laterals are in the process of fusion and there are five median bundles. The two families Calyceraceae and Goodeniaceae have advanced a step further: the venation in the corolla consists of five median and five fused lateral bundles. This is the condition which, represented in the Calyceraceae by *Boopis anthemoides* Juss. (fig. 7), is primitive for the Compositae, as illustrated by *Helianthus divaricatus* L.

In macroscopic appearance the venation of the ray corolla is so distinctly different that a grouping of the tribes according to three types may be made. The tribes which have ray florets fall into three well-defined groups which are termed the "Aster," "Heliantheae," and "Mutisieae" types. A fourth group includes tribes having florets all alike, tubular and perfect; it may be termed the "Discoid" type.

I. Aster Type

Tribes: Asterae, Inulae (*Inula*), Helenieae, Anthemideae, Senecioneae, Calendulae, Arctotideae, Mutisieae, Cichorieae.

In the *Asterae* and *Inulae* (*Inula*) the strap-shaped corolla has four bundles. These bundles represent four fused pairs of laterals. The fifth fused pair of laterals, which is present in the disk corolla, has been lost in the ray corolla. The loss has occurred either through direct reduction of one of the five bundles, or by the abortion of the bundle lying in the position for the split in the corolla. It is believed that the latter process provides a line of weakness which is related to the split in the corolla. The discussion of this point will be made when the anatomy of the floret is presented. When the four bundles of the strap-shaped corolla reach the sinuses the laterals divide and pass into the corresponding lobe. In *Aster paniculatus* Lam. and *Aster novae-angliae* L. the remnants of a bundle are still to be seen in the expanded corolla. This bundle may be interpreted as the fifth bundle that is in process of loss through reduction. In *Erigeron canadensis* L. there are only two bundles remaining in the expanded corolla of most florets although corollas with three or four bundles, or the vestiges of these bundles, may be found in other florets in the same head. *Tussilago Farfara* shows extreme reduction of the veins in the corolla in having only one bundle. *Grindelia squarrosa* (Pursh) Duval. has six bundles. That there are six here, instead of the five to be expected, is because of the lack of fusion of one pair of laterals above the split in the corolla. This is also the bundle situation in the ligulate corollas of the *Cichorieae*. The presence of five bundles in the corollas of *Aster laevis* L. and *Inula viscosa* L. is due to the separation of the two members of one fused lateral at a position in the corolla that is not associated with the split in the corolla. Thus these five bundles are not homologous with the five bundles present in the disk corolla of *Chrysanthemum Leucanthemum* L. (fig. 4). The evidence for this will be discussed below. The venation of the strap-shaped corolla of the *Mutisieae* is similar to that found in the *asterae* (fig. 12).

Tagetes erecta L. and *Helenium autumnale* L. suggest the venation of the *Heliantheae*, but the lack of the large appressed bundles, which will be discussed under Type II, distinguishes the *Helenieae* from the *Heliantheae*. In *Tagetes erecta* there are ten, and in *Helenium autumnale* four, five, or more bundles.

The venation in the *Anthemideae*, *Calendulae*, *Arctotideae* and *Mutisieae* is similar to the four-bundle type characteristic of the *Asterae*. The *Senecioneae*, however, show an interesting reduction from a type with ten bundles, as in *Arnica longifolia* (DC.) Eaton, through six bundles, as in *Arnica Griscomi* Fernald, five bundles typically in *Tussilago Farfara*, three bundles typically in *Petasites palmatus* Presl., to one bundle in some of the corollas of *Tussilago Farfara* and *Petasites palmatus*. Thus we find in this one tribe types which relate the complete story of the reduction in the bundle system of a composite corolla. Vascular reduction is clearly coincident with the gradual reduction in the size of the corolla.

II. Heliantheae Type

Tribe: Heliantheae.¹

In the ray corolla of *Bidens cernua* (fig. 14) and in other allied genera there are eight or more typical veins and two or three other prominent large veins which differ from the first group. These large veins are rather distinct from the rest of the veins of the corolla. They are surrounded by loose parenchymatous tissue, and are, as it were, appressed to and fused with the corolla tissue. There is no indication of an epidermal layer separating the large veins from the corolla proper, but there is a marked distinction between the tissue of the corolla and the tissue surrounding an "appressed vein." These large veins appear clearly as accessory structures. A discussion of the ray corolla in the Heliantheae will be given at a later date. A complete anastomosis of the typical veins is formed at the tip of the corolla.

This net work of veins is exactly what is found in the corolla of an "Aster" type. In the "Heliantheae" type, however, there are, in addition, the large veins which lie on the outer surface of the corolla and extend to the very end of the corolla.

III. Mutisieae Type

Tribe: Mutisieae.

Bilabiate, two lipped corolla (fig. 13). The corolla of this type consists of a strap-shaped (ligulate) lobe and two shorter lips which spread laterally. The corolla is tubular at the base. There are five bundles in the tube. Two of these bundles pass up into the strap-shaped lobe undivided, and three of them divide at the sinuses, furnishing each of the two shorter lips with two bundles. Therefore, the four bundles in the strap-shaped lobe represent two bundles that have come from the base of the flower (two pairs actually), and two bundles that were formed by the division of the bundles at the sinuses between the strap-shaped lobe (ligule) and the two shorter lips (individual laterals). It is evident that the large lip represents three of the usual five lobes and each of the side lips, one lobe. The corolla is not peculiar except in the depth and irregularity of the lobing.

IV. Discoid Type

Tribes: Vernoniaeae, Eupatorieae, Inulae (except *Inula*), and Cynareae.

These tribes contain the genera that have their flowers all alike, tubular and perfect. The venation is identical with that of the typical disk corolla (fig. 4).

In the Vernoniaeae and Eupatorieae the bundles divide at the sinuses and pass to the corresponding lobes of the corolla.

In *Antennaria*, a member of the tribe Inulae, the corollas show two kinds of venation:

¹ Classification according to Engler and Prantl, *Natürlichen Pflanzenfamilien*.

1. Veins dividing immediately below the sinus, as in *Antennaria dimorpha* (Nutt.) T. & G.
2. Veins dividing at different levels in the tubular region of the corolla, as in *Antennaria plantaginifolia* (L.) Richards.

RELATION OF COROLLA TYPES

The Discoid type shows the five fused lateral bundles dividing at the sinuses, the branches running along the margins of the corresponding lobes and uniting at the apex (fig. 4). The disk corolla of all tribes possess this type of venation and also the florets of tribes such as the Eupatorieae and Vernonieae. This is the pattern for the venation of the corollas of the Compositae. The Aster type usually shows the four-bundle condition, but a reduction or increase in the number of bundles is common (figs. 8, 9). A venation consisting of four bundles is derived from the five bundle venation of the disk corolla through the loss of one of the bundles usually in the position where the corolla splits. The Asterae and Inulae may be cited as examples. Ray corollas possessing five bundles do not represent in their corollas the five fused laterals of the disk corolla, but three fused laterals and the separation of one pair of these laterals to make the fourth and fifth members of the group. Many genera of the Senecioneae have a venation which represents the division of the fused laterals and the successive branching of the bundles. The corolla of the Heliantheae type has a number of small, typical bundles, and two or three very prominent ones. The prominent bundles are surrounded by loose parenchymatous tissue that is appressed to the corolla tissue. This is the distinctive feature of practically all of the corollas of the Heliantheae, and clearly sets this tribe off from all other tribes of the Compositae. In the Mutisieae type the venation of a typical disk corolla with deep divisions of the fused laterals at these points gives another variation of the Compositae corolla. The strap-shaped portion of the corolla represents three lobes, and the two lateral extensions form the other two lobes.

The study of the gross morphology of the various types of venation found in the corollas of the Compositae does not give a basis upon which a study of the derivation of the types can be made. Tribes that are considered taxonomically advanced may possess a primitive type of venation, that is, a corolla containing ten or more bundles, as in *Senecio Douglasii* and *S. Pseudo-Arnica*. On the other hand, tribes that are considered primitive taxonomically, as the Astereae, for example, are advanced from the point of view of the reduced number of veins in the corolla. In *Aster* the four bundle situation is generally found, while in *Erigeron*, another member of the Astereae, the corolla contains but one bundle. Both cases show reduction in the number of bundles, but in *Erigeron* reduction has reached the extreme. A study of the venation in the corollas makes it possible to trace the transition in the venation from a primitive type to an advanced

type, but it does not show the origin of the vascular system, nor does it suggest whether a basic similarity exists between the ray and disk corollas. This question must be answered by the study of the floral anatomy of the ray and disk florets.

ANATOMY OF THE DISCOID FLOWER

Aster laevis will be used as an example of the anatomy of the disk corolla. A single bundle, constituting the floral stele, enters the flower from the receptacle (Pl. LX, fig. 16). Five branches from this bundle move out toward the periphery of the ovary wall (fig. 17). The remaining vascular tissue supplies the ovule (fig. 18). Very weak additional bundles arise in the ovary wall at this level, but terminate abruptly (fig. 19). The five original bundles appearing at the base of the flower persist, and when continued above the ovary constitute the five fused laterals of the corolla. From two of these five original bundles the dorsal carpellary bundles, which supply the pistil, are derived (fig. 20). The two dorsal carpellary bundles are evidently fused to two of the corolla bundles as far as the top of the ovary. At this point the fusion ceases and a bundle passes into each style. Such fusion and the departure of bundles at various levels is to be expected, as the wall of an inferior ovary, in most cases at least, represents the fused basal portions of all floral organs, and the vascular supply of these organs gradually tends to fuse and become compound. In other disk flowers examined in the same genus, one dorsal carpellary bundle is adnate in this fashion, the other bundle free. This is due to the position of the carpels in relation to the corolla bundles, only one being directly opposite a bundle. The calyx exists as a remnant in this group and shows no vestigial bundles (fig. 21). At this level the five corolla bundles move inward and take their place in the proximal corolla tissue which is being formed. The bundles of the filaments are adnate to the corolla bundles and remain in that position until the anthers bend away from the corolla proper. At this level (fig. 22) the corolla spreads outward, and in cross section the corolla bundles (fused laterals of adjacent petals) and the anther bundles are opposite each other and alternate with the lobes of the corolla.

In the disk and ray corollas examined no instance of an upper distributive center at the top of the ovary such as Small (27) describes was found (figs. 31, 32). Each bundle is independent of the other at the top of the achene and no series of anastomoses forms the basis for the distribution of the corolla bundles. In the Heliantheae what may be mistaken for a distributive center will be discussed later.

In the disk corolla of *Solidago nemoralis* Ait. there are nine bundles given off from the floral stele. Three of these nine bundles gradually weaken and drop out, and one is a dorsal carpellary. Thus before the calyx region, the top of the achene, is reached the five corolla bundles are in position for the expansion of the corolla. If we recall that the primitive

disk corolla has five median bundles and five fused lateral bundles, the presence of the bundles which die out can be accounted for, these being perhaps vestigial corolla supply bundles; or they may, however, be vestigial calyx bundles and one may be a dorsal carpellary bundle. The majority of disk flowers examined showed the weak bundles alternating with the strong persisting bundles.

The Eupatorieae and Vernonieae present a venation that is similar to the one described for the disk corolla of *Aster laevis*.

ANATOMY OF THE RAY FLOWER

The bundle arrangement in the ovary wall of the ray flower presents practically the same story as is found in the disk corolla. The corollas of some ray flowers of *Aster laevis* have four bundles, while others have five. Both of these conditions are common and may be found in corollas in the same head. A four-bundle ray corolla will first be described, and the five-bundle type will be compared with it. As in the disk corolla, a trace enters the flower from the receptacle. This trace, the floral stele, branches, forming eight bundles. Six of these bundles are strong; two are weak and end shortly (figs. 33, 34). Of the six main bundles two are dorsal carpellary, in this instance, not adnate to corolla bundles. This condition of the carpellary bundle is not constant. In other florets of the same species that were examined one or both of the dorsal bundles was found adnate to the corolla bundles. A ventral carpellary bundle supplies the ovule. The apparent second bundle seen in the ovule (fig. 24) is the same bundle cut in its later course since the ovule is anatropous. As is to be expected, this bundle ends in the ovular tissue. At this level the dorsal carpellary bundles begin to move from the ovary wall towards the center and take their place in a median position in the style. The corolla bundles move inward and the remnant of the calyx disintegrates. As in the disk flower, no vestigial calyx bundles were found. There are now four bundles supplying the corolla. These four bundles extend into the expanded ray corolla. The corolla opens in a region which was weakened by the loss of a bundle. The radius occupied below by a dorsal carpellary bundle is the position in which the corolla splits. Possibly the weakening of the corolla in this way is related to the splitting.

What is the situation in a ray corolla of *Aster laevis* having five bundles? As the floral stele breaks into its strands five traces are given off. These five bundles persist in the ovary wall until the top of the achene is reached. One of the five bundles ends abruptly, the other four continuing into the corolla tissue. The dorsal carpellary bundles which supply the style are fused with two of the corolla bundles. At the level at which the corolla tissue emerges and the base of the style is formed, the two dorsal carpellary bundles move towards the center of the floret. In the corolla tissue at this level there are now four bundles. The fifth bundle was lost before the

corolla tissue was formed, and in the radius of the corolla above the position of this fifth bundle, the split in the corolla occurs. But in the expanded ray corolla there are five bundles. This fifth bundle is not a bundle that would compare with the vascular supply in a disk corolla, but a bundle that was a member of one of the four fused laterals. The lateral that gives rise to this branch is not necessarily identical in all corollas. It is clear that here, as compared with the disk corolla, one of the five fused corolla laterals is lacking. It is in the position of this lost bundle that the corolla opens. In *Aster laevis* the abortion of a vein weakens the corolla tissue and at that point the division is made more easily.

In other ray corollas having five bundles, instead of the trace dividing into five strands, only four strands are formed. The fifth strand in this instance has been entirely lost. The sequence of the vascular supply to each floral whorl is the same as described above. The split in the corolla comes in a position on a radius that lies over the lost fifth bundle. In the expanded corolla the presence of a fifth bundle is due to the separation of the members of one of the four fused laterals.

Ray corollas of other tribes that show the four and five bundle situation in the corollas were examined. *Senecio aureus* L. and *Solidago nemoralis* Ait. were chosen, because the former has a five bundle venation, the latter, four bundles, and because both species grow abundantly in the Ithaca region.

In *Senecio aureus* (figs. 33-36) the trace at the base of the flower divides into five strands. One of these strands persists for a short time only. The dorsal carpellary bundles are adnate to the corolla bundles: neither one is independent, and at the level at which the calyx ends, these bundles split away and take a median position in the style (fig. 34). There are now four bundles in the corolla tissue, and the fifth bundle, which appears in the expanded corolla, is derived from one of the fused laterals (fig. 35). However, the split in the ray corolla does not come between the division of a fused lateral, but as in *Aster*, in the position of the fused lateral which has been lost (fig. 36).

In *Solidago nemoralis* Ait. the trace divides into eight strands, four of which end abruptly (fig. 39). The position of the bundles in the ovule and the adnation of the dorsal carpellary to the corolla bundles, is similar to the condition found in *Senecio aureus* and *Aster laevis*. Again, the split in the corolla lies in the position of the aborted bundle (fig. 40).

In some florets of *Erigeron canadensis* the floral stele divides into four strands. One of the four bundles ends in the ovary wall, thus reducing the number of bundles to three. The expanded ray corolla contains three bundles. The split in the corolla is not in the position of the fourth bundle that has aborted, but in the position of the fifth fused lateral bundle that is lacking. This is understood only when the bundle supply of a disk floret is used for comparison.

An example of extreme reduction is seen in *Tussilago Farfara* (figs. 37, 38). One bundle persists in the corolla of some florets, although four strands were given off from the floral stele. Two of the strands end abruptly in the wall of the ovary. Of the remaining two strands, one is a dorsal carpellary bundle fused to the one bundle retained in the corolla, and the other bundle is a second dorsal carpellary. The one-bundle type is, however, the more reduced form in the species, for corollas with four bundles occur in the same head with the one-bundle corollas. Of the material examined, no instance of one strand given off from the floral stele was found. Reduction of the bundle system is evident in the corolla and in the ovary wall, but not at the base of the floret. The four strands given off as the floral stele branches are still retained. In *Aster* and *Solidago*, four bundles persist through the ovary wall and are continued into the corolla. In *Erigeron* and *Tussilago* the floral stele divides into four bundles, but in these species only one or two of the bundles are retained in the corolla, the others are lost at various levels in the ovary wall. The vascular reduction in the ray floret can be traced from corollas with a supply of five bundles to corollas with a supply of only one bundle. The disk corolla with its vascular supply of five bundles is the type which has been modified as the ray corolla evolved. The first change was the termination of one of the bundles in the ovary wall in a position that was associated with the split in the corolla. Then followed the abortion of the bundle which reduced the number of bundles at the base of the floret to four. The retention of four bundles at the base of the ray floret is still found in all ray florets of the "Aster" type. Some ray corollas of the "Aster" type show an increase in the number of bundles in the corolla. This is due to the separation of the original four fused bundles as the corolla expands. The number of bundles at the base of the floret has not changed. Even in the most reduced types of ray corollas the number of strands given off at the base is four.

Some genera have ray corollas containing six bundles. In the Liguliflorae, for example *Lapsana communis* L., there are five strands given off from the floral stele. As in the disk corolla, these strands persist through the ovary wall. At the top of the achene the two dorsal carpellary bundles separate from two of the corolla bundles. The style is supplied by these bundles (fig. 41). The stamen bundles remain adnate to the corolla bundles until the level of the formation of the filaments (fig. 42). The split in the corolla occurs between two petals, and the fused lateral at that point divides (fig. 43). The anatomical make-up of the floret is similar to that of a disk floret until the level of the split in the corolla. At this point the division of a fused lateral increases the number of bundles in the corolla to six.

In the Cichorieae the corolla of *Centaurea Cyanus* L. is fundamentally of the Discoid type. The corolla has become enlarged and elaborated in its upper region, but the venation is that of the typical disk flower (fig. 10).

The five fused laterals persist through the wall of the ovary and into the tube of the corolla. Here the laterals separate, then branch, to supply the accessory lobing in the corolla. In corollas that have seven and eight prominent lobes secondary branches from the laterals lead into the accessory lobes. The split in the corolla comes at a point where a fused lateral vein divides. The Cichorieae form the example in the Compositae where reduction in the bundle system and decrease in the size of the corolla have not occurred.

A basic anatomical similarity is found in the ray and the disk florets. In both types of florets the disposition of the vascular supply at the base of the achene, the fusion of the bundles in the wall of the ovary, and the giving off of the stylar supply are identical. The normal five bundles of the disk corolla are represented in the ray corolla. In the ray florets of the "Aster" type, vestigial stamen bundles are not present, but certain genera in the Heliantheae still retain them. The most evident change in the gross morphology in the disk corolla has been affected in the ray corolla through the splitting of the gamopetalous corolla. There are two ways in which the splitting of the corolla may take place. In the Cichorieae and the Cynareae the division of a fused lateral bundle is followed by the split in the corolla. In the typical ray of the "Aster" type the abortion of one of the fused lateral bundles provides a line of weakness which is related to the splitting of the corolla. Evidence of this nature shows clearly that the ray corolla is a modification of the disk corolla, and those fundamental structures which are present in the disk corolla are still to be found in the ray corolla.

SUMMARY

1. A primitive gamopetalous corolla of five petals usually has a vascular supply of fifteen bundles, ten of which are lateral bundles and five are median bundles. The venation in a primitive corolla of a disk floret of the Compositae consists of ten bundles, of which five are fused lateral bundles (pairs of bundles) and five are median bundles. This is observed in *Helianthus divaricatus*.

2. A series of disk corollas may be found to illustrate the reduction from the primitive condition (fifteen bundles) to a type with five bundles. In the reduction the median veins are lost, and the fused laterals are shortened, their branches lost, and they terminate at the sinuses.

3. A five-lobed gamopetalous corolla with the lateral bundles in the process of fusion may be found in the Rubiaceae. Such a form shows how the lateral bundles in the Compositae may have been fused phylogenetically.

4. There are three types of venation in the ray corollas of the Compositae: "Aster," "Heliantheae," and "Bilabiate." The "Aster" type usually has four bundles in the expanded corolla, but in some forms there may be five or more. The increase in number of bundles is brought about by the separation of the members of the fused laterals and the subsequent

branching of these members. The "Heliantheae" type has eight or more typical veins and two or three other prominent large veins which differ from the first group. The "Bilabiate" type is found in the Mutisieae. The corolla consists of a strap-shaped lobe and two shorter lips which spread laterally. The members of three fused laterals separate at the three sinuses to supply the ligulate lobe and the two lips.

5. A fourth group, the "Discoid" type, includes all forms in which the corolla consists of five fused laterals dividing at the sinuses, running along the corresponding lobes and finally uniting at the apex of each lobe.

6. *Aster laevis* is used as an example of the anatomy of the disk floret. Five to ten strands, given off from the floral stele in the base of the disk floret, furnish the vascular supply of the floret. Five bundles always persist and are the fused laterals of the corolla. The stamen and stylar bundles are adnate to the corolla bundles.

7. The venation of the ray corolla originates in the same manner. One of the bundles aborts before the top of the ovary is reached; thus only four bundles constitute the supply to the corolla. In cases in *Aster* and *Senecio* where there are five bundles in the corolla, the extra bundle represents the member of a fused lateral that has separated in the corolla.

8. The reduction in the bundle system of a ray corolla from five bundles to one bundle may be traced in *Aster laevis* and *Erigeron canadensis*. The same number of bundles is given off from the floral stele in each example, but in *Erigeron canadensis* there is the retention of only one or two of the bundles in the corolla. Extreme reduction of the bundle system to one bundle in the corolla is found in *Tussilago Farfara*.

9. The split in the ray corolla takes place at the point where a fused lateral bundle has aborted or divided. This provides a line of weakness which is related to the splitting of the corolla. Ray florets of the "Aster" type have evolved from disk florets in which there has been the abortion of one of the bundles in the wall of the ovary. The split in the corolla occurs in a radius of the corolla above the aborted bundle. The other type of splitting is brought about by the division of one of the fused laterals. The Cichorieae and Cynareae show this method for the splitting of the corolla. In the Mutisieae three fused lateral bundles separate at three sinuses which split the corolla into three distinct lobes.

10. The corolla of the Cynareae is elaborated and enlarged. The lobes of the corolla number seven, eight, or more. Branches from the five fused lateral bundles in the tube of the corolla supply the accessory lobes.

11. The ray corolla is a modification of the disk corolla and shows the same fundamental structure as is found in the disk corolla.

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DESCRIPTION OF PLATES

PLATE LIX

FIG. 1. *Hamelia patens*. (Rubiaceae.) Diagram of the vascular supply in a five-lobed rubiaceous corolla showing the lateral veins in the process of fusion. $\times 5$.

FIG. 2. *Senecio Fremontii*. (Compositae.) Diagram to show a stage in the loss of the median veins in a disk corolla which contains ten bundles. $\times 10$.

FIG. 3. *Helianthus divaricatus*. Diagram to show the primitive venation (five fused laterals, five median bundles) in the disk corolla of the Compositae. $\times 10$.

FIG. 4. *Chrysanthemum Leucanthemum*. Diagram of the venation in a typical disk corolla of the Compositae to show the fused lateral bundles dividing at the sinuses and running along the margins of the corresponding lobes. $\times 15$.

FIG. 5. *Xanthium orientale*. Diagram of the venation in the disk corolla to show reduction in the vascular system. The bundles that border the margins of the lobes have been lost. $\times 15$.

FIG. 6. *Anastrophia ilicifolia*. Diagram of the gross venation in the tubular corolla of the Mutisieae to show the primitive type of venation. The five median bundles are still retained and there are five fused lateral bundles. $\times 5$.

FIG. 7. *Boopis anthemoides*. (Calyceraceae.) Diagram of the type of venation in the Calyceraceae. It is similar to the primitive type of venation found in the Compositae (five fused laterals and five median bundles). $\times 10$.

FIGS. 8 and 9. Diagrams of the gross venation in the ray corolla of the "Aster" type. $\times 10$.

FIG. 10. *Centaurea cyanus*. Diagram of the lobing and venation in a tubular corolla of the Cynareae to show the method by which the accessory lobes receive their vascular supply. $\times 5$.

FIG. 11. *Lapsana communis*. Diagram of the gross venation in a ligulate corolla. One of the five fused laterals has divided in the position where the corolla splits. $\times 10$.

FIG. 12. *Gerbera Anandria*. Diagram of the venation in the ray corolla of the Mutisieae to show its similarity to the ray corolla of the "Aster" type. $\times 10$.

FIG. 13. *Perezia microcephala*. Diagram of the venation in a bilabiate corolla of the Mutisieae to show the division of the fused laterals at three sinuses to supply the strap-shaped lobe and the two lateral lips. $\times 10$.

FIG. 14. *Silphium perfoliatum*. Diagram of the gross venation in a ray corolla of the Heliantheae to show the three prominent bundles appressed to a venation that is typical of the ray corolla of the "Aster" type. $\times 10$.

FIG. 15. *Bidens cernua*. Diagram of gross venation in a ray corolla of the Heliantheae to show the three prominent bundles and the lack of anastomosis of the veins in the typical venation. $\times 10$.

PLATE LX

FIGS. 16-22. *Aster laevis*. Diagrams of cross sections of disk floret. FIGS. 16-18. The floral stele breaks into five strands. FIG. 19. A trace from the floral stele supplies the ovule. FIG. 20. The supply to the pistil is fused to two of the corolla bundles. FIG. 21. At the top of the achene the calyx disintegrates. FIG. 22. The five stamen bundles separate from the corolla bundles.

FIGS. 23-26. *Aster laevis*. Diagrams of cross-sections of ray floret with four veins. FIGS. 23-24. The floral stele breaks into nine strands, one of which supplies the ovule. FIG. 25. The bundles which supply the pistil are not fused to the corolla bundles. FIG. 26. The corolla splits in a position in a radius that lies over an aborted bundle.

FIGS. 27-30. *Aster laevis*. Diagrams of cross-sections of ray floret with five veins. FIGS. 27, 28. The floral stele divides into four strands. The supply to the pistil is fused to two of these strands. FIG. 29. One of the fused lateral bundles separates. The venation in the corolla consists of five bundles. FIG. 30. The split in the corolla occurs in a radius that lies over an aborted bundle.

FIG. 31. Diagrammatic longitudinal view of venation in ray floret of "Aster" type. $\times 10$. The corolla splits in a radius that lies over an aborted bundle.

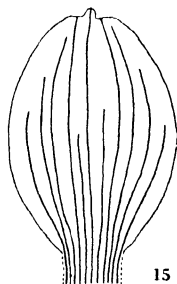
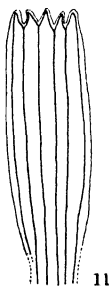
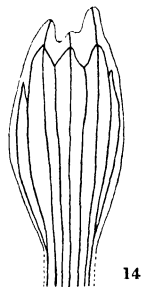
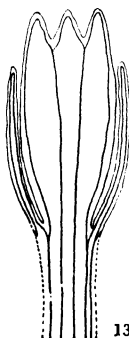
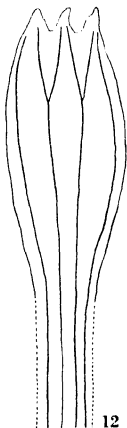
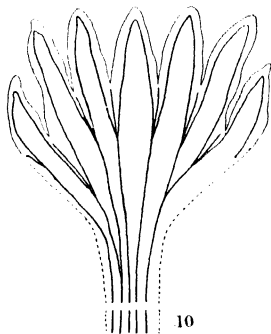
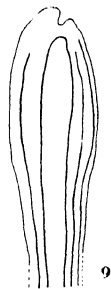
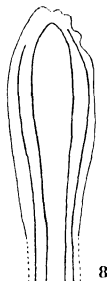
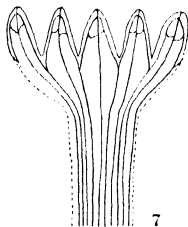
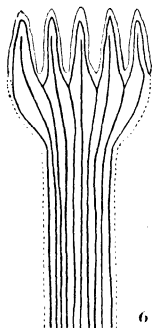
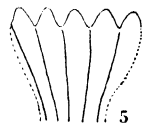
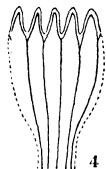
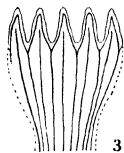
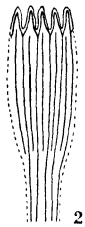
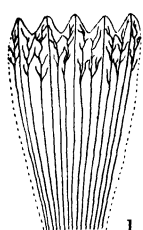
FIG. 32. Diagrammatic longitudinal view of venation of corolla in a disk floret of the Compositae. The supply to the corolla consists of five bundles.

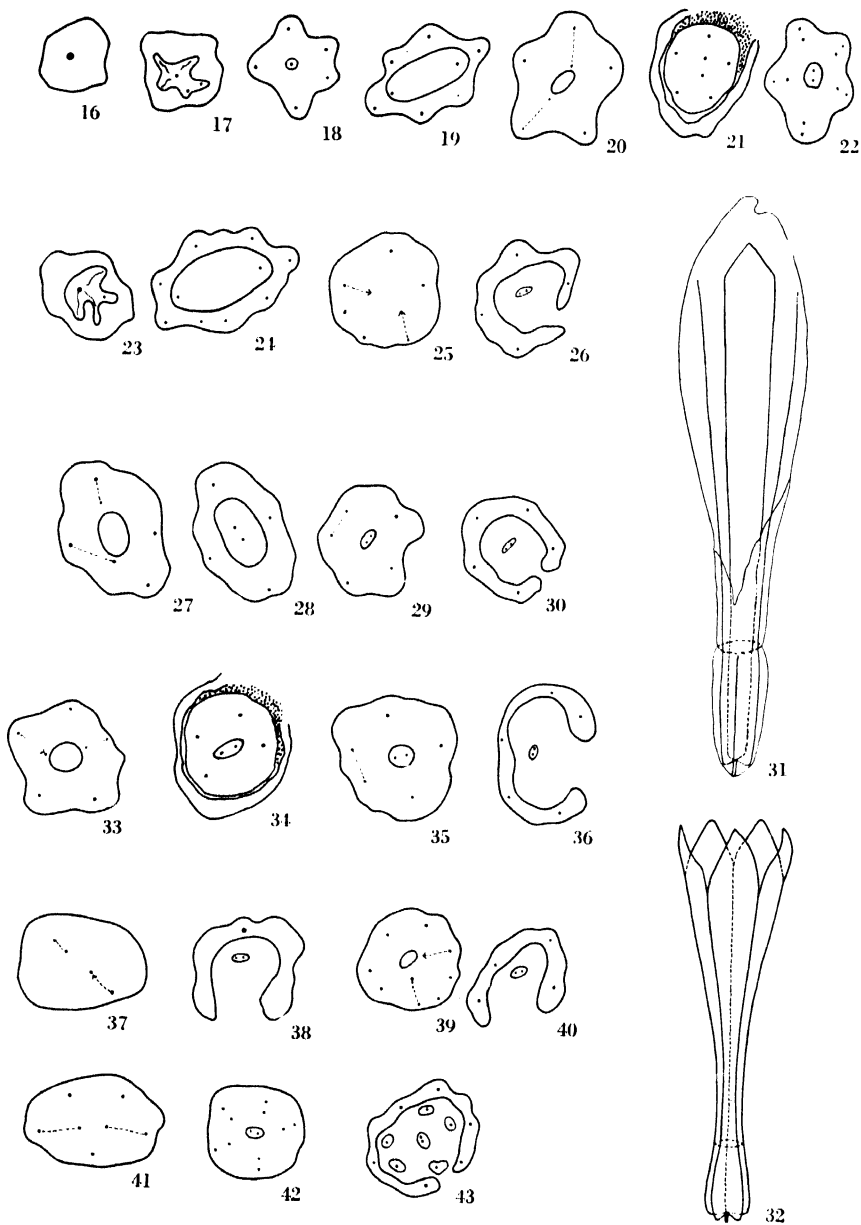
FIGS. 33-36. *Senecio aureus*. Diagrams of cross sections of ray floret. FIG. 33. One stylar bundle is fused to a corolla bundle, the other is free. FIGS. 34, 35. The remnant of the calyx disintegrates, and one of the corolla bundles divides. FIG. 36. The corolla splits in a radius that lies over an aborted bundle.

FIGS. 37, 38. *Tussilago Farfara*. Diagrams of cross-sections of ray floret with one bundle to show extreme reduction in the vascular system.

FIGS. 39, 40. *Solidago nemoralis*. Diagrams of cross-sections of ray floret. FIG. 39. There are vestigial bundles in the wall of the ovary. FIG. 40. The supply to the corolla consists of four bundles which represent four fused laterals. The fifth fused lateral, as is found in the disk corolla, has aborted. The split in the corolla lies in a radius above the aborted bundle.

FIGS. 41-43. *Lapsana communis*. Diagrams of cross-sections of venation in Liguliflorae. FIG. 41. The stylar bundles are fused to two of the corolla bundles. FIG. 42. The stamen bundles separate from the corolla bundles. FIG. 43. The split in the corolla comes between two lobes and a fused lateral divides at that point.





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LIGHT AND THE PERMEABILITY OF PROTOPLASM

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INTRODUCTION

More than twenty years ago my experiments showed that the photonastic movements of leaves of Leguminosae are produced by changes in the permeability of protoplasm which bring about corresponding changes in the turgor pressure of pulvinus cells. In these cells the increase of the permeability of protoplasm produced by light was proved by three methods. The first consists in a determination of the amount of substances which diffuse into water from the pulvini. According to the second method, the concentration of the cell sap in slices which were immersed in water was observed. Due to the great permeability of the protoplasm of pulvinus cells this concentration decreases very quickly. The third method was that of isotonic coefficients. The results obtained by all three methods were similar. The permeability of protoplasm was found to be 1.2 to 1.8 times greater in diffuse sunlight than in the dark. The protoplasm of pulvini presents no exception. Indeed, further experiments showed that it is very probable that every kind of protoplasm changes its permeability under the influence of light; not only the protoplasm of pulvini which are known as most sensitive to the changes of light, but also the protoplasm of *Spirogyra* and of the epidermis cells of *Tradescantia discolor* (*Rhoeo discolor*) increases its permeability in light (1908, 1909 a, b).

This observation was confirmed later by Tröndle (leaf cells of *Tilia* and *Buxus*, 1910), Blackman and Paine (*Mimosa*, 1918), Segel (*Elodea* and other water plants, 1918), Packard (*Paramecium*, 1925), Lvoff (stomata cells, 1926), and recently by Brooks (*Valonia*, 1926) and Hoffmann (*Spirogyra*, 1927). Moreover, the supposition that light increases the permeability of protoplasm has been said by several authors to explain best the results of their investigations.

At the same time, I know of only three papers in which the conclusion was expressed that light may not necessarily increase the permeability in all

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cases. It is easy to show, however, that this conclusion is not proved by the facts presented. I should like to mention first the paper of Efimoff (1925), who found the absorption of methylene blue by cells greater in light than in the dark. He found it less in light, however, when he used vesuvine and toluidine blue, while he could not see any difference in coloring when he used neutral red. As is explained below, this result was brought about by the fading of dyes in light, by the absorption of the most active rays by some dyes, and sometimes by the poisonous effect of the latter.

The second paper the results of which apparently contradict my conclusion is by Kisselew (1925). This author came to the conclusion that the cells of stomata of some plants behave differently from those of other plants, showing an increase of the permeability of protoplasm in the dark. Lvoff (1926) pointed out, however, that Kisselew would not have come to this conclusion if he had considered the work of Arends upon the changes of turgor pressure in stomata cells. The chief method used by Kisselew in his experiments was the observation of the starch dissolution in the stomatal cells after the epidermis had been immersed in solutions of potassium nitrate. Some authors believe that this dissolution (hydrolysis) may be produced by neutral salts, although this opinion seems to be incorrect (see, for instance, Malishev, 1929). Kisselew thought that the breaking down of starch in the stomatal cells was produced by the penetration of potassium nitrate. At the same time, he observed that this proceeds more quickly if the stomata are closed, and concluded that potassium nitrate penetrates more easily in the dark. Arends showed that the open stomata close quickly after an immersion in water or watery solutions, and that the sugar of their cells is transformed thereby into starch. Indeed, in Kisselew's experiments the open stomata immersed in solutions of potassium nitrate did not show at first any hydrolysis of starch, and the number of starch grains even increased while the stomata were closing. If the stomata had been closed, Kisselew of course could not observe this process. His conclusion is therefore incorrect. Lvoff rightly pointed out that Kisselew's experiments merely confirmed the results obtained by Arends, and that Kisselew could not discover any changes of permeability by his method. Thus, Kisselew's observations that the formation of starch in the stomata cells, after the immersion of the epidermis in sugar solutions, proceeds more quickly in the dark merely proves once more that light checks the transformation of sugar into starch. The fact that the stomata cells do not differ from other cells and show a greater permeability of protoplasm in light than in the dark, was proved by Lvoff's experiments.

The third paper which endeavors to prove that not every kind of protoplasm changes its permeability under the influence of light was made in Fitting's laboratory by Zycha (1928). He came to the conclusion that there is no distinct increase of the permeability of protoplasm in light in the case of the epidermis of *Rhoeo discolor*.

It is well known that ten years ago Fitting opposed the method of isotonic coefficients used in some of Tröndle's and my experiments to determine the permeability of protoplasm. I showed, however, that his objections have neither theoretic nor experimental basis (1909 *b*). This method was used afterwards by several investigators with good success (as, for instance, by Ruhland, 1925) and even Zycha used the same method. It is evident that Fitting himself recognized the usefulness of the method of isotonic coefficients. In a previous paper it was pointed out that the exactness of Fitting's plasmolytic experiments is in general insufficient. Zycha used, as an object for this experiment, the epidermis of *Rhoeo discolor*. This requires the determination of isotonic coefficients made on the same slice or at least on a few slices which must be transferred from the isotonic salt-peter solution into the sugar solution. Zycha did not fulfill this requirement, but determined the isotonic concentrations by using two parallel rows of slices. Such a method is not exact enough in this case, because the concentration of the cell sap of epidermal cells is comparatively small and varies too much, while the permeability of protoplasm is not great enough. It is therefore not surprising that Zycha was unable to observe the difference between the isotonic coefficients in light and in dark.

Thus the author's conclusion that the increase of the permeability of protoplasm produced by light is a general property of protoplasm seems never to have been successfully refuted. Every kind of protoplasm possesses selective permeability which is caused by its peculiar chemical and physical structure. It would therefore be strange if this permeability should be influenced by light differently in different plants.

This increased permeability of protoplasm applies to all substances dissolved in water since the presence of water in protoplasm is the only cause of the penetration of water-soluble substances through it. As nutritive substances are mostly soluble in water, the influence of light on permeability is evidently very important for life, and should be investigated in detail.

MATERIAL AND METHODS

There are several methods for the determination of the permeability of protoplasm, but not all of them can be used in experiments made to establish details as to the influence of light. As changes of permeability occur rapidly only those methods can be used which permit a determination during a period of time which does not exceed two hours. The method must also allow an illumination of the cells during the determination of the permeability. Thus, the chemical method (the determination of the amount of the substances diffusing from tissues), which often requires a considerable time, can be used only if the permeability is exceptionally great. On the other hand, the method of electric conductivity of tissues used by Osterhout does not permit the illumination of the cells well enough. This method

may be used only in the case of suspended cells. Therefore, the most suitable methods for the investigation of the details of light effects on multicellular organisms are the plasmolitic ones and the method of the absorption of aniline dyes. In the experiments described in the present paper the last was used and the objects were leaves of *Elodea densa* and *E. canadensis* which, being water plants, are well suited for investigations requiring an immersion in watery solutions.

The same materials and method were used twelve years ago by Segel under the direction of the writer, in her experiments on the influence of different agents upon the permeability of protoplasm. Brooks employed the same method but on *Valonia*.

Before a description of the experiments upon the influence of light it will be necessary to consider some peculiarities of the method of the absorption of aniline dyes. It should first be remembered that according to Pfeffer, who introduced this method into cell physiology, aniline dyes form some compounds with the substances of the cell sap which can not diffuse outward and are therefore accumulated in the latter. These compounds may be decomposed by water, but this process is very slow, though it can be accelerated by acids. The substances forming these compounds with aniline dyes are present in the cell sap in so great an amount that the formation of the compounds proceeds almost instantaneously, at least at the beginning of the process. The speed of the penetration of this dye into the cell sap depends therefore only upon the speed of penetration through the protoplasm, that is, upon its permeability.

All cells composing the *Elodea* leaf may accumulate dyes but this is especially great in several dozens of cells scattered throughout the lower layer of the leaf. These cells do not contain chloroplasts, but more of a substance absorbing aniline dyes (probably tannin) than do the rest of cells, and their protoplasm is more permeable, which results in a quicker accumulation of the dye. Segel observed separately the coloring of these cells and the cells surrounding them. The tint of both was compared in her experiments under the microscope with the tint of a special series of capillary tubes filled with solutions of the same dye. The tint of different cells of both kinds is not alike, however. In order to avoid an error in the determination of the intensity of the tint, I preferred to compare the complete leaves, which had been immersed in a dye solution, with a special scale prepared of leaves colored by the same dye, thus avoiding a use of the microscope. This scale for methylene blue was prepared in the following manner:

About 35 leaves cut from the same part of an *Elodea* stalk were immersed into a 0.0025 percent solution of methylene blue. After the lapse of given time, single leaves were taken from the solution in succession, dried by blotting paper, fastened to object slides in a successive series, and covered by glass. The first leaf of the scale remained $\frac{1}{4}$ minute in the dye solu-

tion, the second $\frac{1}{2}$ minute, the third 1, the fourth $1\frac{1}{2}$, the fifth 2, the sixth $2\frac{1}{2}$ and the seventh 3 minutes. After that the time intervals increased 10 percent each time; the 8th leaf remained therefore 3.3 minutes in the isolation, the 9th 3.6, the 10th 4, the 11th 4.4, the 12th 4.8, and so on. Such an increase of time intervals corresponds to the Weber and Fechner law of perception; the difference in the tint of two neighboring leaves of the scale was therefore approximately the same. The difference in coloring between the first leaves of the scale is greater, because the addition of the green color of chlorophyll to the weak blue tint makes the difference between the tints indistinct.

The above scale may be used for some days, but when its leaves die (in one or two hours), they can be compared only with dead (dried) leaves, colored in the experiments. The scale must be prepared from leaves of the same plant as the leaves used in the experiments, and the tint must be even, without spots.

The tint of the leaves of the scale expressed in the number of minutes during which they had been immersed in the dye solution is proportional to the amount of the dye which the leaves contain, because the absorption of the dye from the same solution is proportional to the time during which they remain in the dye solution, at least during the first 25 minutes of absorption. This can be seen from the following experiment:

Solutions of methylene blue of different concentrations were successively poured into a square narrow vessel made of object slides (8 cm. long, 3 cm. high, inside width 4.49 mm.). One side of this vessel was covered with tin-foil in which an opening was cut out in shape and size similar to the leaves of a freshly prepared scale. One untinted leaf cut from the same plant was placed on the outside of the glass wall of the vessel within the opening just mentioned and was illuminated by diffuse sunlight passing through the solution of the dye in the vessel. The tint of this leaf was compared with the tint of the leaves of the scale which was covered by tin-foil with openings corresponding in size, shape, and situation to its leaves. The results of the comparison are given below. C is the concentration of methylene blue in the vessel; T the tint of the scale leaves (expressed in minutes of their immersion in the 0.0025 percent dye solution), found to be equal to the tint of the uncolored leaf illuminated by the light passing through the dye solution in the vessel; R the ratio C/T multiplied by 10^6 .

| | | | | |
|-----------|---------|---------|---------|---------|
| C | 0.0008% | 0.0004% | 0.0002% | 0.0001% |
| T | 23.8 | 11.1 | 5.2 | 2.5 |
| R | 34 | 36 | 38 | 40 |

Average ratio $C/T = 37/10^6$.

As the substances of the cell sap gradually become saturated with the diffusing dye, further absorption decreases and finally stops (in about $1\frac{1}{2}$

hours, using 0.0025 percent methylene blue). In my experiments 0.0008 percent dye solutions were used, and the leaves were never kept for more than two hours in these solutions. We may therefore admit that the speed of absorption of dyes and the amount of the accumulated dye were proportional to time. That allows us to calculate the amount of dye penetrating into an *Elodea* leaf during a unit of time, and through a unit of surface of the leaf if we know to what leaf of the scale it corresponds in its tint. The ratio of this amount to the concentration of the dye in the surrounding solution is equal to the diffusion coefficient of the dye through protoplasm or to the permeability of protoplasm to the dye.

If it is found, for instance, that the tint of some leaf in an experiment is equal to the tint of the scale leaf which had been immersed in the solution of methylene blue (0.0025 percent) during T minutes, we may conclude that both leaves contain the same amount of the dye. This amount will be equal, in grams, to $(0.449 \times S \times 37 \times T/10^6) \div 100$, where 0.449 is the thickness of the above vessel in cm., S is the area of the leaf, and $37 \times T/10^6$ the concentration of the dye in the vessel in percent. In my experiments the leaves were always immersed for two hours in the dye solution (0.0008 percent). As the dye penetrated from both sides into the leaf, and the area of protoplasm through which the dye penetrated into the cell sap is approximately equal to the total area of the leaf, that is, $2S$, the permeability of protoplasm to the dye is approximately equal to

$$\frac{0.449 \times S \times 37 \times T}{10^6 \times 100 \times 2S \times 0.0008 \times 2} = \text{about } 5 \cdot T \cdot 10^{-5}$$

(expressed in centimeters, hours, grams, and percent).

If for instance, the tint of a leaf in an experiment is found to be equal to that of the scale leaf which had been immersed in 0.0025 percent solution of methylene blue for three minutes, the permeability of protoplasm to this dye is $15 \cdot 10^{-5}$. In all figures given in this paper the permeability should be multiplied by 10^5 .

In order to compare the permeability of protoplasm for aniline dyes in light and in the dark, Segel used to cut the leaf to be investigated along its vein and to color one half of this leaf in light and the other half in the dark. My experiments showed, however, that this precaution is not necessary. If the plant had been cultivated under good and constant conditions, the leaves of the same stalk and of about the same age are colored similarly. The difference in the tint of different leaves does not surpass the difference between the tint of the neighboring leaves of the scale. In the few cases when this difference was greater the average permeability was used.

I usually cut 20-50 leaves situated at the same part of a branch of the plant. Every sample of material investigated under special conditions contained five leaves. They were put into a specimen tube containing 10 cc. of the dye solution and closed by a cork stopper. The concentration of

0.0008 percent was selected because the adsorption of the dye on the cell walls is too distinct if the concentration is greater, while weaker solutions require more time to color the leaves sufficiently. Water used was from the water mains; its pH was always adjusted to 7.2, and it contained about 0.002 percent Ca. After two hours in the solution mentioned protoplasm showed a perfect rotation, and the colored cells bore plasmolysis and deplasmolysis very well.

Many aniline dyes are destroyed not only by direct but also by diffuse sunlight. The solution of dyes exposed to sunlight was therefore usually changed every fifteen minutes. In case of special sensitiveness, the solution of the dye was allowed to flow through the tube containing the leaves with a speed of several cc. per minute. In order to prevent the warming of the dye solutions exposed to direct sunlight, the tubes were immersed in a water bath through which water flowed constantly.

THE CAUSE OF THE DIFFERENT ABSORPTION OF DYES IN THE LIGHT AND IN THE DARK

Investigators who have studied the absorption of aniline dyes by plant cells in light and in dark admit that the observed difference in absorption is due to the change of the permeability of protoplasm. In my experiments, the tint of leaves was compared with the tint of scale leaves. One may object, however, that the difference in coloring might be produced by the change of the adsorption of the dye on the cell walls or by a difference in absorptive power of the cell sap in light and in the dark. Both objections are refuted by the following experiments.

Fifteen leaves of *Elodea canadensis* were put into a 0.0008 percent solution of methylene blue in three tubes, five leaves in each tube. One of the tubes was exposed to direct sunlight, another was left in diffuse light, and the third tube was wrapped in black satin (three layers). The second portion, consisting of fifteen leaves, was first killed by a diluted solution of iodine, then washed in water and put into the dye solution, in three tubes. One of these tubes was exposed to direct sunlight, the second was left in diffuse light, and the third was wrapped in black satin. After a lapse of two hours, the tint of the leaves corresponded to the tint of the scale leaves which had been immersed in the dye solution (0.0025 percent) during *T* minutes, as follows:

| | 1st Portion (Living Leaves) | | | 2d Portion (Dead Leaves) | | |
|---|-----------------------------|------------------|------|--------------------------|------------------|------|
| | Direct Sunlight | Diffuse Sunlight | Dark | Direct Sunlight | Diffuse Sunlight | Dark |
| T | 9.2 | 7.7 | 3.6 | 12.2 | 12.2 | 12.2 |

Only living leaves show a different absorption of the dye in the light and in the dark. The adsorption on the cell walls therefore can not be at all important in the change of absorption.

Observations under the microscope showed that dead cells absorb the dye by their protoplasm and chloroplasts, while the cell sap remains colorless (exosmosis of tannin), with the exception of the above tannin cells scattered among the cells of the lower layer. These cells evidently contain substances which do not diffuse outwardly even after death.

The second objection, that light may change the absorptive power of the cell sap, is refuted by the following experiment:

One portion of leaves of *Elodea densa* was immersed in a 0.0025 percent solution of methylene blue and remained in diffuse sunlight, while another portion of leaves of the same plant in the same dye solution was placed in the dark. After nine minutes the tint of the leaves of the first portion was found to be equal to $T = 9.2$, the tint of the second portion was $T = 7$. After 22 minutes, the difference in tint was still distinct, but after the cell sap had been saturated by the dye (90 to 100 minutes), the difference disappeared completely. Further immersion of the leaves in the dye solution did not increase their tint. We may therefore conclude that the maximal absorption of the dye after the saturation of the cell sap by it does not depend upon illumination. Thus, the difference in the absorption of the dye in light and in the dark is due exclusively to the change of the permeability of protoplasm.

THE ABSORPTION OF DIFFERENT DYES IN THE LIGHT AND IN THE DARK

It was mentioned in the introduction that according to Efimoff only methylene blue is absorbed in light more markedly than in the dark. Such a result was obtained by this author only because he did not consider the chemical changes of aniline dyes produced by light. All dyes which showed an abnormal behavior in Efimoff's experiments are especially sensitive to light. I exposed, for instance, 0.0008 percent solutions of vesuvine, toluidine blue, and neutral red to direct sunlight for half an hour. The concentration of toluidine blue (colorimetrically measured) was decreased by this exposure four times, that of neutral red three times, and that of vesuvine at least ten times. The chemical structure of the dyes was also changed. Neutral red, for instance, did not become violet after the increase of pH to 6.0, while vesuvine was no longer brown but yellow. Even the concentration of methylene blue exposed to direct sunlight for half an hour was decreased about twice.

Diffuse sunlight did not affect the dyes so markedly but its action was quite distinct. After an exposure for four hours to diffuse light (on a window sill, on the north side of the building), the concentration of the same solution of vesuvine decreased two and a half times, that of neutral red about twice, and that of toluelene blue more than twice.

We see, therefore, that experiments with the absorption of aniline dyes by living cells in light will give correct results only if the solutions of dyes are often changed or flow through the vessel where the objects are exposed

to light. Indeed, if these conditions were not fulfilled, an opposite result was always obtained: the leaves exposed to direct sunlight showed a weaker coloring. On the contrary, if the dye solution flowed first through illuminated tubes, and then through a tube wrapped in black satin, the leaves exposed to light always showed a stronger coloring. Only in the case of vesuvine was the difference not distinct, but even in this case I never observed a stronger coloring in the dark, as Efimoff observed. The indistinct difference in the case of vesuvine may be explained, first, by a weak absorption of this dye by *Elodea* leaves and second, by the absorption of the most active violet rays of visible light by the solution of vesuvine (see below). Indeed the addition of vesuvine to the solution of methylene blue decreased the coloring of the leaves exposed to light.

The best way to prove that light increases the permeability of protoplasm to aniline dyes is to use the method common in experiments with the formation of starch in leaves. In using this method, I fastened two strips of tin-foil, three millimeters wide, on an object slide at a distance of two centimeters from each other, laid three leaves of *Elodea* (*canadensis* or *densa*) crosswise on each strip, and fastened them with soft bees'-wax at the tip and base. Then the object slide with leaves was put into the dye solution (0.0008 percent) which flowed through the tube during the experiment. The tube was exposed to direct sunlight for two hours and care was taken that tin-foil strips shaded the leaves. The illuminated parts of the leaves were colored much more markedly than the shaded parts.

In making the above experiment, one must take care that the exposure to direct sunlight does not last too long in the case of poisonous dyes. My previous papers (1923, 1927) showed that light decreases the resistance of protoplasm against harmful effects, and if the dye is poisonous, it kills the cells in direct sunlight. The least poisonous is methylene blue, and it can be used for the experiments described above without hesitation, while toluidine blue often kills the cells. At the same time, the killed parts of the leaf are colored more markedly than the shaded living parts.

DIFFERENCE BETWEEN THE PHOTOTROPIC REACTION AND CHANGES OF PERMEABILITY PRODUCED BY LIGHT

According to Tröndle, who tried to establish certain details as to the action of light upon the permeability of protoplasm, the change of permeability represents a reaction similar to the phototropic reaction. The so-called law of the amount of energy observed in phototropism can also be applied, according to Tröndle, to the changes of permeability produced by light. He could not discover, however, any similar dependence of the relative change of the permeability upon the amount of light. He gives a curve expressing the dependence of the time necessary to produce a change of permeability on the intensity of light, and finds it similar to the curve expressing the dependence of the phototropic reaction upon the intensity

of light. Moreover, Tröndle finds that the change of permeability depends upon the intensity of preliminary illumination, and concludes that light influences the "mood" of the plant.

It is well known that every phototropic phenomenon consists of the perception of the stimulus, the conducting of this stimulus to the place where the movement occurs, and the movement itself. On the contrary, in the case of the change of permeability produced by light, the place of the perception of stimulus and of the reaction is the same. Indeed, it was mentioned that the increase of the permeability can be observed by shading a part of the leaf by a strip of tin-foil. The investigation of the obtained photogram produced on the leaf shows that the boundary between the illuminated and shaded parts of the leaf is very sharp; the permeability increases only where light is perceived by the cells, and no transmission of the stimulus is observed.

Concerning the similarity between the curves found by Tröndle, it should be remembered that both curves, light intensity on illumination time (phototropism) and light intensity on reaction time (permeability), will express the same law only if the reaction, that is, the change of permeability, is always the same. One may doubt, however, that this took place in Tröndle's experiments, because the method used by him does not allow of a determination of the moment when the change of permeability begins, and when the change is observed one can not be sure that in all experiments it is of the same strength. Actually Tröndle found only that the weaker the light producing the change of the permeability, the slower this change proceeds. But such a law is common to all photochemical processes. I believe that not only the change of permeability produced by light, but also the phototropism, at least in its first part (perception), represents a photochemical process, but I doubt whether both processes are the same.

It is therefore very likely that light acts directly on the permeability of protoplasm, decomposing some substances (or substance) in it which participates in the creation of the selective permeability. If the changes produced in protoplasm by light were only some processes which induced another process resulting in the change of permeability, we might expect that this change would not occur immediately after the change of illumination, but would require some time to become distinct. If we let light act on the leaves of *Elodea* interruptedly, periods of illumination following periods of darkness, we should expect that permeability would continue to increase during the periods of darkness, and would be found greater if both kinds of periods were long than if they were short, supposing that the amount of light received by the leaves in both cases is equal. My experiments showed, however, that permeability remains the same in both cases. This may be seen from the following.

Two portions of *Elodea* leaves were put into the solution of methylene

blue, and exposed to direct sunlight diminished to 20 percent by rotating discs (made of cardboard) in which two sectorial openings were made, each with an area of 10 percent of the whole area. One disc rotated 400 times in one minute, the other disc only once in two minutes. After two hours the permeability was found to be equal in both cases.

Thus light acts directly on the permeability of protoplasm. Only the amount of light energy received by the cells is important, and the manner in which this energy is received may be different.

THE DEPENDENCE OF THE PERMEABILITY OF PROTOPLASM UPON THE INTENSITY OF LIGHT

It was mentioned that Tröndle found the change of permeability to depend upon the intensity of the preliminary illumination. He also found that the illumination by an electric lamp of 32 candles at a distance of 10 cm. from the plant produced first an increase of permeability, but then a decrease of it if the illumination continued for more than one hour. He concluded from this observation that permeability does not depend upon the intensity of light (Tröndle, 1910, p. 192), but he admitted that the changes of permeability are produced by the changes of illumination. Such a conclusion may be ascribed only to the incompleteness of his experiments. For instance, he determined the permeability only once a day (p. 203), or during four hours. In his experiments described on p. 196, the permeability was not determined during the period of time between four and 24 hours after the beginning, and so on.

In my experiments the permeability of protoplasm was investigated for several days and determined oftener than in Tröndle's experiments. In one case the leaves were kept in uninterrupted light, in another in uninterrupted darkness. A short account of these experiments follows. Both experiments were made at Long Beach, California.

I. At 12 o'clock noon ten leaves were cut from a stalk of *Elodea canadensis* (cultivated in diffuse sunlight on the window-sill) and put into the solution of methylene blue in two tubes, five leaves in each; one tube was left in diffuse sunlight, while the other was wrapped in black satin. After two hours the permeability of the leaves was determined. At the same time the same stalk of *Elodea* was placed (in water) in a dark room and left there for several days. Four or five times a day a portion of leaves was cut from the stalk and put into the solution of methylene blue, and after two hours the permeability was determined. The results are given in table 1. Permeability is expressed in units specified above. Temperature was 24° C.

II. At 8 o'clock in the morning another stalk of the same plant of *Elodea* used in experiment I was left in uninterrupted light produced by an electric bulb of 40 watts at a distance of 15 cm. from the plant. Between the bulb and the crystallizing dish in which the stalk was immersed in water a thick glass was inserted. Moreover, water was changed every three hours

TABLE 1

| Date | Time | Conditions of Illumination | Permeability |
|---------------|---------------|----------------------------|--------------|
| July 12 | 12 to 2 P.M. | Light | 21 |
| | 12 to 2 " | Dark | 15 |
| | 2 to 4 " | " | 10 |
| | 4 to 6 " | " | 5 |
| | 8 to 10 " | " | 15 |
| July 13 | 9 to 11 A.M. | " | 15 |
| | 11 to 1 P.M. | " | 7.5 |
| | 1 to 3 " | " | 7.5 |
| | 3 to 5 " | " | 10 |
| | 6 to 8 " | " | 15 |
| July 14 | 10 to 12 A.M. | " | 7.5 |
| | 1 to 3 P.M. | " | 5 |
| | 4 to 6 " | " | 5 |
| | 8 to 10 " | " | 10 |
| July 15 | 9 to 11 A.M. | " | 5 |
| | 11 to 1 P.M. | " | 5 |
| | 3 to 5 " | " | 7.5 |
| | 8 to 10 " | " | 7.5 |
| July 16 to 18 | | " | 5 to 7.5 |

to prevent the rise of temperature. Three or four times a day a portion of leaves was cut from the plant and put into the solution of methylene blue. The permeability is shown in table 2.

TABLE 2

| Date | Time | Permeability | Date | Time | Permeability |
|---------|-------------------|--------------|---------|---|--------------|
| July 16 | 8 to 10 A.M. | 28 | July 17 | 8 to 10 A.M. | 26 |
| | 11 A.M. to 1 P.M. | 24 | | 11 A.M. to 1 P.M. | 26 |
| | 1 to 3 P.M. | 24 | | 6 to 8 P.M. | 26 |
| July 18 | 9 to 11 A.M. | 18 | July 19 | 9 to 11 A.M. | 26 |
| | 4 to 6 P.M. | 26 | | 11 to 1 P.M. | 26 |
| | 8 to 10 " | 26 | | At 1 o'clock the plant was transferred into the dark. | |
| | | | | 2 to 4 P.M. | 12 |

From the experiments cited above one may see that the permeability of protoplasm may vary a little in continuous light or dark but that it always remains smaller in the dark than in light. The variation of the permeability in the dark seems to be a periodical one, but it continues only the first three days, after which permeability becomes almost constant. This reminds us of the variation of the turgor pressure in pulvini after the transferring of the legume plants into the dark. It is very probable that a marked decrease of the permeability causes a process in the cell which finally leads to an increase of permeability.

Experiments on the changes of permeability brought about by the changes of the intensity of illumination will now be described. The preliminary illumination should also be considered.

The plant to be investigated was cultivated on the window-sill on the

north side of the building. The illumination there was about 50 times as weak as the direct sunlight (estimated by photographic chlorine-bromine paper). In order to use only a part of direct sunlight, I partly shaded the sun by quickly rotating discs (made of cardboard) in which sectoral openings of different size were cut out so that the leaves shaded by these discs received amounts of sunlight ranging from 10 to 90 percent. In all experiments the tubes with leaves were put into metal boxes open on one side and immersed in flowing water; the solution of methylene blue flowed through the tubes constantly with a speed of 5 cc. in one minute. For the shading of the leaves I sometimes used ordinary white paper in which the tube containing the leaves and the dye solution were wrapped. The paper transmitted about 2.5 percent of sunlight. A short account of my experiments follows.

I. The leaves cut from the plant at 10 o'clock in the morning were divided into three parts. One part remained in diffuse sunlight (the tube was wrapped in white paper), the second part was transferred to the dark (the tube was wrapped in black satin) and the third part was exposed to direct sunlight. At 12 o'clock all leaves were put into tubes with the solution of methylene blue. One tube with the leaves of the first part was exposed to direct sunlight, one remained in diffuse light, and one was transferred to the dark. One tube containing the leaves of the second part was exposed to direct sunlight, one remained in the dark, and one was transferred to diffuse sunlight. One tube containing the leaves of the third part was transferred to the dark, one remained in direct sunlight. After two hours the color of the leaves was determined, and the results are given in table 3. Temperature was 24 to 27° C.

TABLE 3

| | First Part of Leaves Left in Diffuse Sunlight, Then Transferred to: | | | Second Part of Leaves Left in the Dark, Then Transferred to: | | | Third Part of Leaves Left in Direct Sunlight, Then Transferred to: | |
|------------------------|---|------|---------------------|--|------|---------------------|--|--------------------|
| | Direct Sunlight | Dark | Diffuse Sunlight | Direct Sunlight | Dark | Diffuse Sunlight | Dark | Direct Sunlight |
| Permeability | 35 | 21 | 25 | 52 | 15 | 21 | 15 | 40 |

From the above experiment, which is one of a series of experiments with the same results, one may see that the intensity of the preliminary illumination is important for the change of permeability. The more this illumination differs from the new illumination, the greater is the change of permeability. Permeability in the dark after diffuse sunlight was found to be 21, while this permeability after direct sunlight was fifteen. Similarly, the permeability in direct sunlight after diffuse light was 35, while this permeability after the dark was found to be 52. This result is in accordance with that of Tröndle's experiments, but instead of being explained by the

change of "mood" of the leaves, as by Tröndle, it can be explained by the supposition that the change of permeability produced by light is a photochemical process and that the cells are able to restore some substances important for the selective permeability of protoplasm and destroyed by light. Both processes (that is, the decomposition and the synthesis) proceed in light, but if the leaves are transferred to the dark, only a restoration takes place. As both processes are chemical, they obey the law of chemical masses. Therefore the more products of the decomposition the protoplasm contains, the quicker the restoration proceeds, and conversely, the more substance is restored in the dark, the quicker proceeds the decomposition. We also have to suppose that the substance (or substances) decomposed by light are present in a limited amount in protoplasm, and that the selective permeability of protoplasm is not annihilated even by a complete decomposition of this substance. Our supposition explains the decrease of permeability after its increase in light, as was observed in Tröndle's experiments and in the experiments cited above. It explains also its greater decrease if the leaves are transferred from direct sunlight to the dark than if the leaves are transferred there from diffuse sunlight, and so on. This supposition is also confirmed by the following two experiments.

I. Twenty-five leaves of *Elodea densa* which had been cultivated in diffuse sunlight were cut at 9 o'clock A.M. and exposed to 10 percent direct sunlight for two hours, then they were divided into five parts and put into the solution of methylene blue. One part was exposed to 10 percent, one to 20 percent, one to 70 percent, one to 100 percent direct sunlight and one was transferred to the dark. The temperature of the water bath was 22° C. The results are given below:

| % of Direct Sunlight | 10% | 20% | 70% | 100% | Dark |
|-------------------------|-----|-----|-----|------|------|
| Permeability found..... | 81 | 81 | 81 | 81 | 16.5 |

II. The leaves cut from the plant used in the preceding experiment were left in diffuse sunlight (2 percent direct sunlight) for two hours, put into the solution of methylene blue and exposed to 10 percent, 20 percent, 70 percent, and 100 percent direct sunlight or transferred to the dark (wrapped in black satin). The dye solution flowed through all tubes containing the leaves with a speed of 30 cc. in one minute. The temperature of the water bath was 22° C. The permeability was found as follows:

| | Diffuse Sunlight | 10% | 20% | 70% | 100% | Dark |
|-------------------------|------------------|-----|-----|-----|------|------|
| Permeability found..... | 38 | 96 | 89 | 89 | 89 | 26 |

From the experiments cited one may see that at 10 percent direct sunlight the maximal increase of permeability is reached, and further increase of illumination does not produce any effect. The substance decomposed by light is evidently completely destroyed by 10 percent direct sunlight. When it is destroyed a further increase of illumination can not increase the permeability; then the restoring process may sometimes decrease the permeability again.

Moreover further experiments showed that the effect of direct sunlight is not always as great as in the two experiments just cited. Sometimes the increase of the permeability in direct sunlight is not more marked than in diffuse sunlight. The substance destroyed by light is evidently contained in protoplasm in a smaller amount in this case. I should like to cite here, for instance, the results of one of my experiments.

Fifteen leaves of *Elodea densa* which had been cultivated in diffuse light on the window-sill were cut at 10 o'clock A.M., five of them being transferred into the dark and the rest remaining in diffuse sunlight (1/50 of direct sunlight). At 11 o'clock all leaves were put into the solution of methylene blue. The tube with the leaves which had been in the dark was wrapped in black satin and the tube containing five other leaves was exposed to direct sunlight. The rest of the leaves remained in diffuse sunlight. Through all tubes a constant flow of the dye solution was arranged with a speed of 15 cc. in one minute. After two hours the permeability was determined. Temperature of the water bath was 23° C. The results are as follows:

| | Diffuse Sunlight | Direct Sunlight | Dark |
|-----------------------------|------------------|-----------------|------|
| Permeability found. | 31 | 46 | 18 |

THE INFLUENCE OF LIGHT OF DIFFERENT WAVE-LENGTHS

Brooks arrived at the conclusion that the greatest increase of the permeability of protoplasm is produced by ultraviolet rays; less effective are blue and green, and the least effective are red rays. My experiments in general confirmed these results, but the difference between the action of red and blue rays was found to be greater than in the Brooks experiments.

I used colored light filters manufactured by Corning Glass Company. The filters were the following: *a*, heat resisting red, G. 24, 2 mm. thick; *b*, light blue green, G. 584, 4 mm. thick; *c*, red purple, ultra, G. 586-A, 3.91 mm.; *d*, corex red purple, G. 986-A, 3.98 mm. The properties of light which is transmitted by these filters are given in table 4.

In order to compare the action of ultraviolet rays I used tubes made of quartz. All tubes were exposed to direct sunlight in metallic boxes open from one side and immersed in water. Each tube contained the solution of methylene blue and five leaves of *Elodea densa*. The temperature of the

TABLE 4

| Name of the Filters | The Part of Sun Energy Transmitted by the Filters (Visible and Ultraviolet Rays only). Approximate Figures | Wave-length of the Rays Transmitted in $m\mu$ | Maximum of of Trans- mission, Wave- length ($m\mu$) |
|--------------------------------|--|---|---|
| Red, G. 24..... | 14% | 600 to 750 | 680 |
| Blue-green violet, G. 584..... | 25% | 350 " 600 | 450 |
| Purple, ultra, 586-A..... | 2% | 320 " 420 | 370 |
| Corex purple, G. 986..... | 4% | 260 " 420 | 340 |

water bath was 23° C. After two hours the leaves were investigated. The permeability found is given below.

| | Dark | White (glass tubes) | White (quartz tubes) | Red, G. 24 | Blue-green, G. 584 | Ultraviolet, 586-A | Ultra- violet, G. 986 |
|-------------------|------|---------------------|----------------------|------------|--------------------|--------------------|-----------------------|
| Permeability..... | 12.5 | 27.5 | 30 | 12.5 | 25 | 16.5 | 18 |

On the supposition that the ultraviolet part of the solar spectrum does not exceed 5 percent of the whole energy (only the visible and ultraviolet parts of the spectrum are considered), we may assume that the most active rays are the ultraviolet rays. This conclusion becomes especially evident if we compare the quotients obtained by the division of the relative increase of permeability (that is the difference between the permeabilities in light and in the dark, $p_1 - p_2$, divided by the permeability in the dark p_2) by the amount of energy transmitted by the filter, e , in percent. These quotients (activity of light) are the following:

| Activity | White (visible) | White (visible and ultraviolet) | Red | Violet-blue-green | Extreme Violet and Ultraviolet, 370 $m\mu$ maxim. | Extreme Violet and Ultraviolet, 340 $m\mu$ maxim. |
|----------------------------------|-----------------|---------------------------------|-----|-------------------|---|---|
| $\frac{p_1 - p_2}{p_2 \times e}$ | 0.012 | 0.014 | 0 | 0.04 | 0.16 | 0.11 |

Although the above figures expressing the amount of energy and the relative activity of single rays transmitted by the filters are only approximate, we may assume that the most active rays in their effect on the permeability are ultraviolet rays with a wave-length of 370 $m\mu$ or near this figure, and that the least active are red rays.

SUMMARY

1. The results of the former experiments of the author which showed that the permeability of protoplasm increases in light and decreases in the dark were confirmed by the investigations of several authors and by the results obtained in the present paper.

2. The results of investigators who endeavor to show that the permeability increases in light only for some substances, while it may decrease in light for other substances, and that the permeability of protoplasm of some plants increases in the dark while it is not changed by the change of illumination in other cases, are incorrect since the methods used by these investigators to determine the permeability were inexact or faulty.

3. In the present paper the influence of light upon the permeability of protoplasm for aniline dyes was investigated. The permeability was measured according to the amount of dye accumulated in the cell sap of *Elodea* leaves, determined with a colorimetric method especially worked out for *Elodea*.

4. The difference in absorption of dyes by plant cells in light and in the dark is caused by the change of permeability of protoplasm. The absorptive power of the cell sap and the adsorption of dye on the cell walls or in dead protoplasm are not changed by light: dead cells absorb dyes with the same speed in light as in the dark, and the maximal amount of dye absorbed by the cell does not depend upon the illumination.

5. The solutions of dyes fade in light, and must be changed during the experiment, or flow through the vessels containing the object. If this condition is fulfilled, the permeability of protoplasm is found to be greater in light to all dyes which penetrate protoplasm and do not absorb violet rays.

6. The increase of permeability is observed only in the leaves or their parts which are illuminated. The increase of permeability does not spread from the cells affected by light even to neighboring cells.

7. The greater is the change of the illumination, the greater is the change of permeability, though no proportionality is observed between these. A chemical explanation of this fact is given. The permeability may increase in light but decrease again afterwards, which is explained by the process restoring substances destroyed by light.

8. For change of permeability only the amount of light energy is important and not the manner in which this energy is received by the plant.

9. The maximal change of the permeability occurs if direct sunlight is diminished to 10 percent of its strength. The further increase of illumination does not produce any effect and the permeability sometimes even decreases a little.

10. The rays most active in producing the increase of permeability are those with a wave-length of 320 to 420 $m\mu$, that is, ultraviolet rays; less active are violet, still less active are blue and green rays, and the least active are red rays.

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THE SWELLING OF CITRUS FRUITS¹

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A consideration of the growth of citrus fruits soon leads to an appreciation of the changes produced by the exchange of water between them and the tree. The water which produces distensive changes in the volume of the fruit comes from the tree on which it is borne. Bartholomew (1926) has shown that there is a sensitive equilibrium between the water in lemon trees and their fruits, which materially conserves the physiological integrity of the tree during periods of stress. The sensitive equilibrium is reflected by diurnal changes in volume of the fruits on trees living in habitats subject to marked diurnal variations in temperature and humidity. A certain amount of water continually escapes from the fruit into the atmosphere through the stomata and even through the cutinized epidermal cells. Exposure to desiccating winds which blew for several days reduced the measured size of immature oranges to an extent that the shrinkage was not overcome for many weeks (Waynick, 1927).

The water transport problem of a fruit like the lemon or orange differs from that of a pomaceous fruit since the fleshy center is not permeated by fibrovascular bundles. The vascular system of the hesperidium is mainly confined to the mesocarp surrounding the fleshy pulp and this layer therefore plays an important role in supplying water to the pulp vesicles. The diverticulae of the ramifying vascular system terminate, not among the pulp vesicles, but among the parenchymatous cells of the mesocarp which must act as channels for the movement of water. These cells are enveloped with layers of pectin and other hydrophilic colloids which appear to act as paths for the facile exchange of water (Reed and Bartholomew, 1927). The rapid changes in volume of lemons previously reported by Bartholomew constitute evidence that water moves in the hydrophilic layers of the cell walls rather than across the protoplasts.

The principal objects of the present investigation were a study of water absorption in the fruit, the concentrations of solutes which affect swelling, and the rate of swelling.

An added reason for the investigation under discussion is the frequent loss of fruit due to the splitting of the peel of oranges and lemons which are approaching maturity. In some seasons the losses in yield of the Navel orange are heavy. The mesocarp generally has the characteristics of

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meristematic tissue and tends to keep pace with the development of the fleshy endocarp. However, the appearance of a higher concentration of solutes in the pulp vesicles produces a higher turgor and the resulting distensive force in some cases is sufficient to split the pericarp layers.

All fruits employed were approaching maturity but were still physiologically active. The fruits were submerged in test solutions as indicated below. The fruits registered any volume changes on the drum of an auxograph in a way similar to that employed by Bartholomew (1926). The pen of the auxograph drew curves having a magnification of 17.5.

The investigation was prosecuted over a period of two and a half years. The experiments were performed in a room maintained at a temperature of $22.5 \pm 1.0^\circ \text{C}$. Fluctuating temperatures produce fluctuations in the absorption of liquids and distention of the fruits which give false values to the records. The records of volume changes were obtained for a total of more than 400 fruits. Each experiment extended over three or more days, although it can be seen from the graphs that the results were usually complete at the end of the second day.

In the first set of experiments the epicarp (epidermis) was removed by rubbing the fruit on a sharp kitchen grater. This treatment equalized the opportunity for uniform absorption by the entire surface of the fruit.

The peel of these fruits showed changes in physical and chemical composition during their periods of immersion in water. The most conspicuous change was the more or less complete hydration of the pectin, but in most instances there was also an escape of substances which reduced Fehling's solution. Table I shows the gain in weight accompanying hydration of disks of peel cut with a large cork borer from an immature orange, December 10. The fresh disks were divided into seven lots of ten each. Each lot was immediately weighed; one lot was put into the drying oven, the others into dishes of distilled water where they were left for various periods as indicated in table I.

TABLE I. *Imbibition of Water by Disks of Orange Peel and the Loss of Solutes Incident Thereto*
(Original Fresh Weight = 100)

| Hours of Immersion in Distilled Water..... | 0 | 1 | 2 | 4 | 6 | 24 | 48 |
|---|-----|-----|-----|-----|-----|-----|-----|
| Weight of the imbibed disks..... | 100 | 231 | 256 | 221 | 250 | 287 | 292 |
| Dry weight of disks after immersion in water..... | 57 | 52 | 51 | 43 | 45 | 38 | 42 |

The figures show a rapid increase in weight during the first few hours of immersion in water. The conditions under which they were when in contact with water were so favorable that the hydration was very rapid. The gain in weight from the 24th to the 48th hour is negligible. In terms of the water relations we may say that the mesocarp of the growing fruit has a very strong water deficit. The non-living colloids on the exterior and the

living colloids in the interior are the principal factors in maintaining an equilibrium between the water of the fleshy pulp and that of the fibrovascular system. Their importance in maintaining the water economy of the growing fruit may be more readily understood from the data which have been given in table 1 and from others which follow.

Table 1 also contains data on the escape of solutes from the disks. Each lot of disks, after the imbibed weight was obtained, was dried in an oven at 70° C. to constant weight. The loss in dry substance which was rather slow in the first few hours eventually amounted to 20 to 25 percent of the dry weight of unleached disks. The loss, as previously mentioned, consisted in part of substances which reduced Fehling's solution and of other constituents which were not determined. Lemon peel contains pentosans and probably pentose sugars (Bartholomew and Robbins, 1926) which, under similar conditions, could be leached out.

Before going further it may be well to discuss a question which will eventually arise, "What does the auxographic graph really register?" When the pen indicates an expansion of the fruit, what has happened? A short, simple answer cannot be given because the fruit consists of many substances which are more or less interrelated. It may assist the reader to understand the situation if we call his attention to the salient features in the morphology of the fruit. The mesocarp at the stage under experimentation consists of spongy parenchyma whose cell walls are thickly overlaid with pectin. Fibrovascular bundles extend through the mesocarp and form an elaborate system of ramifications. The central mass of endocarp consists of locules which are filled with juice vesicles each of which is a glandular hair whose stalk is attached to the inner face of the tangential wall of the locule. The locule walls are cellulose. The juice vesicles are filled with a solution containing sugars, acids, and salts.

It is apparent that there is here a system of cell walls having a capacity for imbibition and a central mass of turgid vesicles which may exert a suction pressure. The turgidity of the endocarp is readily seen if one makes an incision around an immature orange or lemon. In a short time the severed edges of the mesocarp will have separated several millimeters. If the fruit after being girdled is laid in water the expansion of the endocarp will be still more marked.

The thick walls and pectin layers of the mesocarp cells may be demonstrated in razor-sections of fresh material. Since the colloids readily absorb water, the outlines of the pectiniferous layers are generally indistinct. It is better to examine the sections in such a medium as paraffin oil or xylol which does not swell the colloids of the cell walls, and in which the thick glistening layers of the cell wall may be seen. The presence of pectin may also be demonstrated by the pink color produced by a solution of ruthenium oxychlorid or by the violet color produced by methylene blue.

In certain plants there may be a loose combination between cellulose and

pectin. Tupper-Carey and Priestley (1923) observed that fresh sections of the meristem of radicle, plumule, and root of *Vicia Faba* were not stained by methylene blue except when they had been treated with reagents which broke up the protein-pectin complex. Sucharipa (1924) concluded that the pectinogen of lemon peel is a combination between cellulose and pectin, since the pectin becomes soluble only after hydrolysis.

This discussion may be summarized by saying that the citrus fruit consists of several elements having marked imbibitional capacities for water and that the change in volume recorded by the auxograph pen is the resultant of changes in the elements of the system which constitutes the fruit. The swelling process of the whole fruit is the correlated system of changes which result from the imbibition and translocation of water among and between these elements. As such it has been studied, though the part played by each element must be recognized in the discussion of the results.

There are certain features of the graphs to which attention ought to be directed. The prompt expansion of fruits in water indicates that there was an unsatisfied capacity for imbibition in most of the fruits. Reference to the graphs shows that in some instances half the expansion of the fruit occurred in the first two hours. When the fruits contracted there was often a brief initial expansion probably due to the more rapid penetration of water than of the dissolved ions. When the ions entered there was often a reversal in the direction of water movement with consequent contraction of the fruit.

BEHAVIOR OF FRUITS IMMERSSED IN LIQUIDS

In nearly every instance the fruit showed a change in volume as registered by the auxograph, indicating that the solvent at least penetrated the mesocarp. The effect of acid, base, salts, and two organic compounds on volume changes are shown in table 2. Sample curves transferred from the auxo-

TABLE 2. *Volume Changes in Fruits Immersed in Solutions*

| Solution | Oranges | | | | | Lemons | | | | |
|--|----------------------------|------------|------------|------------|-------------|----------|------------|------------|------------|-------------|
| | Concentration of Solutions | | | | | | | | | |
| | <i>N</i> | <i>.5N</i> | <i>.2N</i> | <i>.1N</i> | <i>.05N</i> | <i>N</i> | <i>.5N</i> | <i>.2N</i> | <i>.1N</i> | <i>.05N</i> |
| HCl..... | < | < | | < | < | > | = | | < | |
| KOH..... | <> | < | < | | < | = | < | | < | |
| CuSO ₄ | < | < | | < | < | <> | | | < | < |
| CaCl ₂ | <> | | <> | <> | | <> | | <> | <> | |
| BaCl ₂ | <> | | <> | <> | | <> | | < | <> | |
| | 50% | 25% | 10% | 2 <i>M</i> | 1 <i>M</i> | 50% | 25% | 10% | 2 <i>M</i> | 1 <i>M</i> |
| C ₂ H ₅ OH *..... | <> | <> | <> | | | < | < | < | | |
| C ₁₂ H ₂₂ O ₁₁ †..... | | | | < | < | | | | < | < |

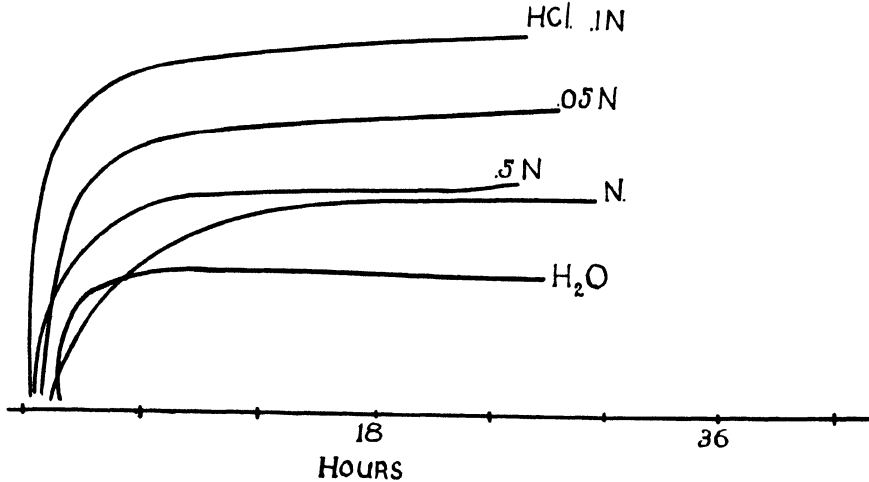
* Concentrations expressed as volume percentages.

† Concentrations expressed as gram-mols per liter.

NOTE: < signifies expansion; >, contraction; and =, no appreciable change.

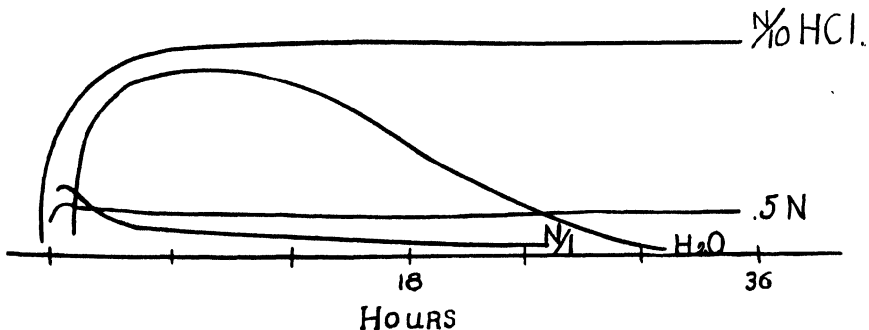
graph charts are shown in the accompanying figures. Space limitations prevent the reproduction of a larger number of curves.

The oranges swelled promptly in all concentrations of hydrochloric acid employed (text fig. 1). The result is noteworthy because all fruits in the



TEXT FIG. 1. Graphs showing the swelling of oranges in hydrochloric acid solutions. The graphs in this and the following figures are magnified 17.5 times.

experiments swelled more than the controls in distilled water. In the normal solution the swelling started slowly but on the second day was as great as that of the fruit in .5N concentration. Hydrochloric acid in concentrations of normal strength has a disorganizing effect on lemon fruits manifested by softening the mesocarp and endocarp. The graphs shown in text figure 2 show permanent swelling in .1N concentration but there was



TEXT FIG. 2. Graphs showing swelling of lemons in hydrochloric acid. The fruit in normal strength acid showed a reversible swelling, that in .1N strength swelled promptly and consistently. The fruit in water showed a reversibility of swelling which is rather characteristic.

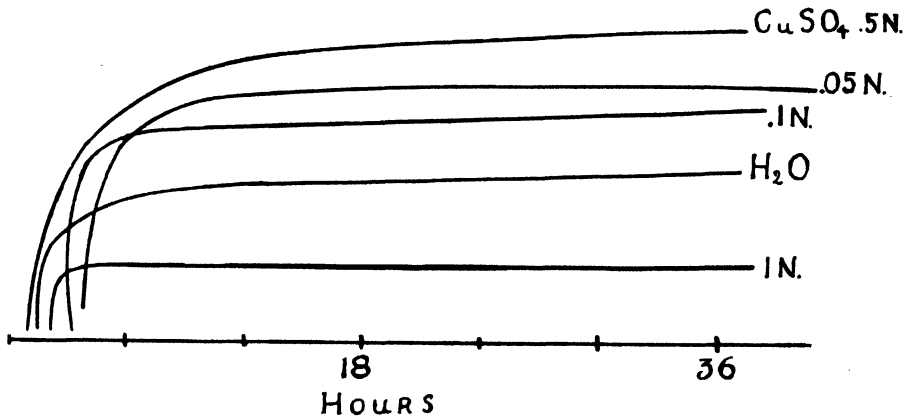
only a slight initial swelling followed by contraction in normal concentration. Oranges immersed in normal KOH showed reversibility of swelling, while

those in lower concentrations of KOH swelled to a volume which was retained for three days. Lemons in normal KOH showed no appreciable change in volume but they swelled in $.5N$ and $.1N$ concentrations.

An examination of the fruits which had been immersed four days in the solutions and the curves from the auxographic charts indicate that concentrations of $.1$ and of $.2$ normal acid or alkali had no disruptive effect on the imbibitory mechanism of the fruit.

Several experiments were made upon the effects of HCl and KOH on the orange mesocarp itself to determine whether the mesocarp was solely responsible for the changes observed. The results of various trials were not unanimously on one side of the question or the other, probably owing to unavoidable fractures in removing the peel, but they showed that the peel itself absorbed KOH more readily than HCl of equi-normal concentration. The control pieces in distilled water showed a distinct swelling during the first six hours followed by a shrinkage at the end of 24 hours, due probably to the disruption of the imbibitory mechanism.

The effects of copper sulfate were remarkable in producing expansion of the fruits with each concentration employed. The normal and $.5$ normal solutions produced less expansion than the weaker solutions employed, but all produced swelling without subsequent shrinkage in volume (text fig. 3).

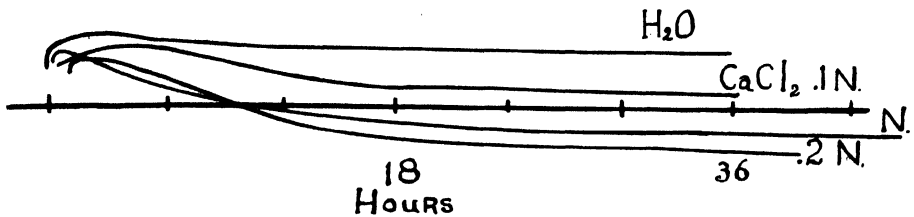


TEXT FIG. 3. Graphs showing swelling of oranges in copper sulfate solutions. The expansion was exceedingly rapid and showed no case of reversal.

Since the solutions employed were toxic to protoplasm, the results may be regarded as the strongest kind of evidence that there was no participation of living cells in the final effect observed.

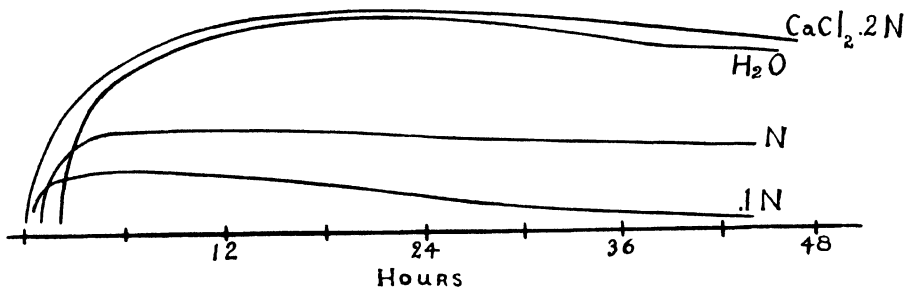
The experiments with solutions of cane sugar were interesting because they employed a solute less toxic than the preceding. Solutions of molecular and half molecular concentrations produced uniform swelling of oranges and lemons without ultimate shrinkage, although sections of the stipe of a marine alga (*Postelsia*) show swelling followed by shrinkage in solutions of less than $.5N$ and show initial shrinkage in solutions of $-N$ strength (Lloyd and Ulehla, 1926).

The experiments with solutions of calcium chlorid and barium chlorid may be considered collectively. It is evident from the symbols in table 2 and from the graphs (text fig. 4) that the effects of various concentrations



TEXT FIG. 4. Graphs showing the incipient expansion and subsequent contraction of oranges in solutions of calcium chlorid.

were essentially similar. There was a brief period in which the oranges expanded, then came a longer period in which the fruits contracted. The lemons, however, had a relatively active period of expansion, followed by a period of contraction (text fig. 5). Fruits in normal solutions of either salt frequently showed a net shrinkage by the end of the third day.



TEXT FIG. 5. Graphs showing swelling of lemons in calcium chlorid solutions. In solutions of .2N and .1N concentration there was a reverse swelling following the early period of distention.

These results have significance for the problem under consideration because calcium and barium form salts with pectic bodies. Whatever the nature of these salts they differ greatly in their capacity for absorbing water from the colloidal compounds on the wall of the mesocarp cells. The net shrinkage of the fruits in these solutions is strong evidence that the layers of hydrophilic colloids on the cell walls are an important path for the absorbed liquids. The contrast in the volume changes of fruits from which the peel had been removed is shown in table 3.

The oranges in this case showed definite expansion in concentrations of .2N and of .1N and contraction in normal BaCl_2 . Decorticated lemons and oranges in CaCl_2 showed dissimilar behavior, but in BaCl_2 they responded similarly to the solutions. The ensemble of the results supports the idea previously stated, namely that the principal path over which the water is transported is through the colloidal layers of the cell walls.

TABLE 3. *Volume Changes of Decorticated Fruits Immersed in Solutions*

| Solutions | Oranges | | | | | Lemons | | | | |
|--|----------------------------|-------------|-------------|-------------|--------------|----------|-------------|-------------|-------------|--------------|
| | Concentration of Solutions | | | | | | | | | |
| | <i>N</i> | .5 <i>N</i> | .2 <i>N</i> | .1 <i>N</i> | .05 <i>N</i> | <i>N</i> | .5 <i>N</i> | .2 <i>N</i> | .1 <i>N</i> | .05 <i>N</i> |
| HCl..... | = | > | <> | < | | c | | | <> | |
| KOH..... | < | < | | < | | | < | | < | < |
| CuSO ₄ | < | < | | < | | <> | | | < | < |
| CaCl ₂ | = | | < | < | | <> | | <> | <> | |
| BaCl ₂ | > | | < | < | | > | | < | < | |
| | 50% | 25% | 10% | 2 <i>M</i> | 1 <i>M</i> | 50% | 25% | 10% | 2 <i>M</i> | 1 <i>M</i> |
| C ₂ H ₅ OH *..... | > | < | < | < | < | = | < | < | > | |
| C ₁₂ H ₂₂ O ₁₁ †..... | | | | < | < | | | | | |

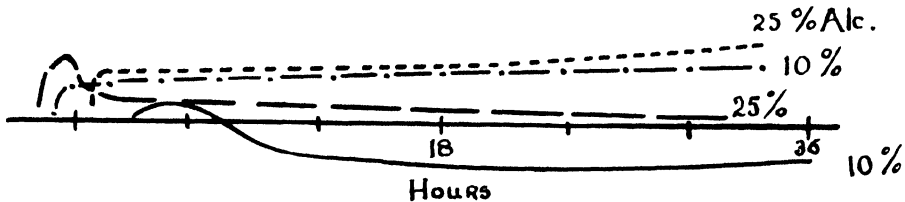
* Concentrations expressed as volume percentages.

† Concentrations expressed as gram-mols per liter.

c. Disintegrated.

NOTE: < signifies expansion; >, contraction; and =, no appreciable change.

The effects of alcohol upon changes in volume of fruits were also ascertained with oranges and lemons since it acts as a precipitant for pectins. At the concentrations used (tables 2 and 3) the oranges (text fig. 6) showed



TEXT FIG. 6. Graphs showing the type of swelling of oranges produced by solutions of alcohol. Solid line, reversible swelling in 10 percent solution; — — —, reversible swelling in 25 percent solution; — · — ·, irreversible swelling of decorticated fruit produced by 10 percent solution; · · · · irreversible swelling of decorticated fruit produced by 25 percent solution.

an initial swelling followed by contraction but the lemons expanded in all concentrations. Decorticated oranges shrunk in 50 percent alcohol but swelled in the lower concentrations. Lemons which had been decorticated behaved like undecorticated fruits except in 50 percent alcohol.

BEHAVIOR OF DECORTICATED FRUITS IMMERSSED IN LIQUIDS

The results obtained by immersing fruits in solutions have been supplemented by additional experiments in which the mesocarp was removed before placing the fruits in the various solutions. The object of these experiments was to learn whether the central endocarp showed the same response to acids, bases, and salts as shown by the fruits enveloped with the

mesocarp layer. The thin membranes which form the locule walls are composed of cellulose which should be readily traversed by solutions. The results of the experiments (table 3) make a valuable addition to those previously presented.

The behavior of decorticated oranges in hydrochloric acid solutions was a contrast to the results with undecorticated fruits. The normal solution produced no change in volume and the half normal produced shrinkage. Solutions of .2*N* and .1*N* strength produced swelling, but the behavior of the former was later reversed.

There was permanent distention, however, in corresponding strengths of KOH. The pectin, pentosans, and associated bodies apparently undergo hydration readily in solutions containing OH ions as MacDougal (1921) pointed out. The failure to swell in acids was not due to lack of penetration of the acids because titration of the juice showed an unmistakable increase in acidity.

The swelling in copper sulfate solutions was of the same order in whole and decorticated fruits. Dissection of fruits which had lain in the stronger solutions for a few days showed that the copper sulfate solutions readily traverse the thin walls of the locules but seldom penetrate the walls of the juice sacs.

The behavior of the decorticated oranges in solutions of calcium chlorid, barium chlorid, and alcohol (table 3) is a point of great interest. In normal solutions of calcium chlorid there was no change in volume. In normal solutions of barium chlorid and in 50 percent alcohol there was shrinkage of the decorticated fruits, but in lower concentrations there was swelling. The swelling process showed no reversibility in any case studied. The reversibility in swelling of whole fruits (table 2) forms a striking contrast, but corresponds with the idea that the change from expansion to contraction was due to the action of the solutes on the pectin-pentosan layers on the cell walls. When the mesocarp had been removed the fruits showed no reversibility of swelling. It will be noted that decorticated lemons showed reversible swelling in .2*N* and .1*N* solutions of CaCl_2 although they were not appreciably changed in volume by the normal solution. The reason for this anomalous behavior is not evident. It may be noted, however, that the whole lemons behaved differently in the three alcohol solutions than whole oranges in similar solutions (text fig. 6).

THE EXCHANGE OF SOLUTES BETWEEN FRUITS AND THE SURROUNDING MEDIUM

Tests were made on the pulp (endocarp) of oranges taken from the solutions of hydrochloric acid after their volume changes had been registered by the auxograph. The object was to determine whether there was a passage of solution beyond the peel into the locules. If so, it may then be inferred that the swelling of fruits registered by the auxograph was due to the expansion of the pulp as well as of the peel.

Three untreated oranges whose volumes averaged 105 cc. were peeled. The pulp was crushed and the juice strained off. The average amount of normal KOH required for neutralization of the juice was 4.5 cc. Three similar fruits which had shown swelling in normal HCl were peeled in similar manner. The juice obtained from the pulp required 4.9, 6.3, and 5.7 cc., respectively, of normal KOH for neutralization (an average of 5.6 cc.). The greater acidity of the fruit in the latter case may be referred to the penetration of HCl into the fruit following the hydration of the biocolloids which envelop the locules.

Dissolved material may escape from the pulp when it is immersed in distilled water. Oranges having an average volume of 75 cc. were taken from dishes of distilled water after the fruits had swelled. The expressed juice required only 2 cc. of normal KOH for neutralization. From the observations on the loss of acid from the fruits and the amount of reducing substance in the bathing solutions, it is evident that there is a transfer of solute in both directions. The fact that the fruits swelled under these conditions is the point of interest, however, and must not be overlooked. It shows that water may move in and through cell aggregates independently of the solutes.

EXPERIMENTS TO SHOW THE ENTRANCE OF WATER INTO THE LOCULES OF THE FRUIT

The following experiments show that the contents of the locules exert a suction pressure which can draw water through the mesocarp and produce a measurable hydrostatic head.

A number of immature fruits, varying in diameter from 5 to 7 cm., were selected and rubbed on a grater to remove the epidermal layer. A sharp cork-borer was thrust into each fruit and when withdrawn it removed a cylinder of tissue extending from the surface to a point well beyond the center of the fruit. Several of the locules were thus cut. A piece of glass tubing with an internal diameter of 8 mm. was inserted in the hole and sealed with chicle. Each fruit was immersed in water, the inserted tube being held upright by a clamp. Distilled water was carefully added to the interior of the tube, bringing the top of the column to the level of the water outside the tube. The results of several experiments are presented in table 4, from which it will be seen that there was a distinct rise of the liquid in the tube in each case.

These results answer a question which has been open to this point, namely, do the aqueous contents of the locules remove water from the imbibed cell walls of the mesocarp? The experiments of Bartholomew and those presented in the foregoing part of this paper show that volume changes readily occur in citrus fruits, but they leave the question open in regard to the entrance of water into the locules. It can now be concluded that the suction pressure in the glandular hairs which fill the locules of the fruit is

TABLE 4. *Passage of Water into Fruits*

| | Height of Water Column in Tube | | |
|---|--------------------------------|-----------|------------|
| | 24 hrs. | 72 hrs. | 96 hrs. |
| A, Orange (hole cut on transverse diameter) | cm. 0 | cm. .5 | cm. 1.0 |
| B, Ditto (hole cut on polar diameter) | 5.0 | 8.0 | 8.5 |
| C, Ditto ditto | 0 | 2.0 | 2.5 |
| D, Ditto ditto | 0 | 2.0 | 2.5 |
| E, Lemon ditto | — | — | 3.0 |
| F, Ditto ditto | — | — | 3.7 |
| G, Ditto ditto | — | — | 2.1 |

great enough to pull water from the hydrophilous colloids which constitute such a large portion of the mesocarp.

CONCLUSIONS AND SUMMARY

1. The structure of citrus fruits presents certain unique problems pertaining to water absorption and conductance since the fibrovascular system is mainly restricted to the mesocarp.

2. Orange and lemon fruits absorbed water and various solutions with resulting increase in volume. Both acid and base were absorbed, but with lemons the latter produced somewhat greater swelling than the acid. Oranges in copper sulfate solutions swelled promptly and showed no reversibility.

3. Solutions of compounds which coagulate pectins caused incipient swelling, but it was followed by shrinkage.

4. The translocation of liquids appears to depend upon their passage through the layers of hydrophilic colloids on the walls of the mesocarp cells and only to a small degree upon the participation of living cells.

5. The juice sacs in the locules of the fruits have sufficient suction pressure to pull water from the hydrophilous colloids in the mesocarp.

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NUTRITION OF THE CULTIVATED MUSHROOM

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The art of mushroom growing has been passed on from generation to generation without the development of exact methods. There is a great and costly lack of understanding of the biological processes which influence this culture. This situation must be charged to the fact that until the last few years the principles of spore germination and spawn production were poorly understood; and students who developed spore germination methods usually entered the spawn business without carrying on further investigations. Although the attitude of the spawn producers toward experimental work has been very helpful to the industry, they have in most cases lacked laboratory facilities for the kinds of work that were needed. Fortunately the development of the industry in America and Germany is leading to a concerted attack on the problems of mushroom growing, by specialists in the lines involved.

This study concerns the growth of the mycelium of the cultivated mushroom on simple media of known composition. The natural habitat of this mushroom is the sod of meadows and plains, but the medium used in mushroom growing is a partially decomposed preparation of horse manure. Chemical studies of this medium were begun by the writer along with this investigation, but the complexity of manures is such that the value of any such analyses is as yet uncertain. The record of growth on simple media throws more light on the nutrition of the organism and forms a more reliable basis for further work.

The literature on the subject of mushroom nutrition was reviewed in a preliminary paper (10). The outstanding work of Duggar (2) was the only one known to the writer in which a study of nutrition was the chief aim. Duggar's cultures were made upon filter paper dampened by nutrient solutions, and the physical nature of the media was supposed to resemble that of manure. His results with a series of carbonaceous materials indicated that the organism preferred starch as compared with sugars, and proteins above all other compounds. Salts of organic acids were apparently of no value. As sources of nitrogen the proteins were best, and growth was uniformly better with ammonium salts than with nitrates. Calcium hippurate seemed useful as a source of nitrogen.

The writer's first study (10) of this problem was carried out on the same plan as Duggar's. The ratio by weight of liquid to paper in Duggar's flasks was about 4 to 1, or 400 percent moisture, while the ratio in the writer's was 2 to 1, or 200 percent, and there was better growth by reason

of better aëration. The importance of aëration as a factor will be shown later in the present paper. The results of this series agreed in general with those of Duggar's, but it was found that the paper base alone could serve as a nutrient. It was also shown that while ammonium salts may be fair sources of nitrogen, the total nitrogen in that form in the medium must be small, about 0.1 molar. Complex organic compounds could be added safely in much larger amount. In both nitrogenous and carbonaceous series the more complex and less soluble nutrients gave the best results.

Careful examination of these results and comparison with Duggar's led the writer to suspect that the poor growth of the cultures upon soluble carbohydrates and nitrogenous compounds might have been due to the higher concentration of those media, rather than to their unsuitability as nutrients.

CONCENTRATION OF MEDIA

In the preliminary study the solution of nutrient salts with which the paper was dampened had a concentration of 0.172 *M*. All cultures in which other soluble materials were added to the extent of 0.1 *M* or over, failed completely. Duggar's solution *A* was made up of salts totaling 0.16 *M*. He added to this solution other soluble materials; for example, 1½ percent dextrose, the concentration of which is 0.08 *M*. In both these series the salts were highly dissociated, further raising the concentration from the osmotic point of view.

A new series of cultures was made to ascertain the effect of concentration, with graded concentrations from 0.1 *M* to 0.3 *M*, and with more than one nutrient balance. It was found that the total concentration must be below 0.2 *M* for vigorous growth, and that the partial concentrations of individual nutrients may be varied without effect on this maximum point. Dissociation of salts, however, must be considered, and in a solution of 0.2 *M* concentration highly dissociated salts may be permitted up to 0.1 *M* only.

This result affords a good reason for the apparently greater value of the less soluble nutrients. The addition at the beginning of soluble nutrients, for example dextrose, might raise the osmotic effect of the medium to a point where the mycelium would not be able to grow at all. The insoluble materials, for example starch, could presumably be added in larger quantity, and be made available gradually by enzym action. This affords also a possible reason for the better growth obtained by Duggar in washed manure. It is a practice of spawn producers to wash the manure used for pure cultures. From the same considerations it would be reasonable to expect that the addition of soluble nutrients to manures used in mushroom growing would harm the spawn. This would depend upon the concentration of the manure solution and the time when the application is made. In Duggar's experiments the results of such additions were negative. Experiments upon manures and other media for mushroom growing, which are becoming numerous among the growers, should be carried out with careful consideration of this important factor.

MOISTURE CONTENT

The growth of the mycelium is indifferent in liquids. The species seems to be intensely aerobic, and too much moisture in fibrous media such as manure will invariably produce an adverse effect upon growth. The danger point in moisture content appears to be the degree of saturation at which free movement of air is cut off.

To determine this degree a number of test-tubes were tightly packed to a depth of 10 cm. with manure which had been washed with distilled water, and distilled water added so as to obtain a series with moisture content ranging from 125 percent to 250 percent of dry weight. The latter figure was the limit of moisture content so that aëration was cut off except through water. The tubes were inoculated at the surface of the medium, and the growth downward was measured. Growth was best at 125 percent, fell off very slowly to 200 percent, and at 225 percent dropped to less than half. With a total volume of manure of 25 cc., the air included at 125 percent moisture was 11 cc., and at 225 percent was reduced to about 3 cc. At saturation no downward growth occurred.

A second experiment in flasks with filter paper and the nutrient solution used in the preliminary investigation (10) gave similar results. This series covered a range from 50 percent to 350 percent moisture content. Growth failed below 100 percent, after a feeble start; it was good up to 233 percent, and then fell off sharply. Aëration was better in the flasks than in the tubes, having been actually cut off only at 300 percent. Aëration in a mushroom bed six inches deep should be good at 200 percent, if, as is usual, the surface is allowed to dry.

It is worthy of note that the nutrition experiments of Duggar discussed above seem to have been carried out with 25 cc. of solution added to each 6 gms. of paper, which would be a condition very unfavorable to growth. Many of his cultures grew only at the surface. The results would probably have been clearer if the moisture content had been better adjusted.

All the cultures affected by high moisture content developed excessive numbers of mycelial strands. These have recently been described at length by Hein (6), and were attributed by him to the stimulus of moist conditions. He stated the least number of strands were produced at a point between 45 percent and 80 percent moisture content; and although the impression was given that the best growth occurred at this point also, that was probably not his intention. There is a tendency to formation of strands in old cultures, and spawn producers are often forced to discard a strain which becomes "stringy." Certain organic nutrients cause strand growth as will later be shown. But if other conditions remain the same, the writer agrees with Hein that increases in moisture result in increases in the number and size of the strands.

Since the strands form profusely in the wetter media, and since they are also essential to fruiting through the translocation of food materials, a

connection between aëration or respiration and fruiting is suggested. The grower maintains his beds dry up to a certain point, and then induces the production of sporophores by watering. It seems probable that while the initial growth is favored by perfect aëration, fruiting is brought about by reduction of aëration, and the addition of moisture is a means of reducing it. Many other factors enter into crop development, but a study of respiration in this connection seems to the writer to have great possibilities.

SILICA GEL MEDIUM

After the preliminary study brought out the fact that the cellulose of filter paper could support growth, and after agar was found to have inherent nutrient qualities, the writer turned to silica gel as an inert solid medium. It is an ideal medium for this type of work, for with surface growth aëration ceases to be a factor requiring consideration. The mycelium can also be observed with ease and comparisons can be made more exactly. The development of this medium has been described in a recent paper (11).

The medium was prepared by mixing two solutions; a potassium silicate solution 0.2 *M* with respect to K_2O , and an acid solution consisting of H_3PO_4 , 0.135 *M*; $MgSO_4$, 0.015 *M*; $CaCl_2$, 0.006 *M*; and $FeSO_4$, trace. For the series of non-nitrogenous nutrients NH_4NO_3 was added to the acid solution in 0.15 *M* concentration. The alkaline solution was poured into the acid solution until a pH of 6.6 was reached, the optimum for this species according to Frear, Styer, and Haley (5), and water added until the volume was one and one-half times the volume of acid solution used. Thirty cc. of this mixture were poured into each plate and allowed to gel. The plates were dialysed in a dishpan full of tap water until about half the soluble materials were removed. The nutrient gel was then of the following composition: $MgSO_4$, 0.005 *M*; $CaCl_2$, 0.002 *M*; $FeSO_4$, trace; potassium phosphates, 0.045 *M*. The gel framework was a 1.2 percent content of SiO_2 , hydrated. When NH_4NO_3 was present its concentration was 0.05 *M*.

After the silica gel plates had been dialysed, the substances whose nutritive value was to be tested were added in finely powdered form. The plates were then sterilized in the autoclave, and inoculated in the center with a small amount of a pure culture on manure. Within reasonable limits no difference in growth has been observed from different sized pieces of inoculum. During sterilization and subsequent standing the soluble substances diffused through the gel, while the others remained as even surface films. The amount of any insoluble material used was adjusted so that the film was not too thick to allow microscopic observation.

SPORE GERMINATION

The cultures used were pure spore cultures, germinated and growing in washed fresh manure. A distinct strain of mushroom called "Snow-white" was used in all cultures. This strain originated in the beds of a Pennsyl-

vania grower; it may be recognized by its failure to develop pigments when grown in light. It is probably a strain of *Agaricus campestris*. No difficulty was met with in germinating the spores, after they had been collected under aseptic conditions. A brief description of the method follows.

Pieces of tissue with gills attached were removed aseptically from vigorous mushrooms before the rupture of the veil, and placed on match sticks in dry sterile Petri dishes. The sporophores naturally contain foreign organisms which may develop on the gills and cut surfaces of the pieces, and may contaminate the spore prints. This was prevented by permitting air to circulate under and around the pieces, which were cut between one and two centimeters square. Plates in which the tissue became damp or moldy were discarded, and the pieces were removed from all plates within 60 hours.

Small squares of sterile filter paper were drawn through the spore prints until heavily coated with spores, and then placed in tubes of sterile washed manure. The tubes were incubated at 25° C., and a mycelium was observed growing out from the spores in from six to ten days.

The quickness of germination and growth in these tubes is a matter of especial interest. The question arises whether the same principles are involved in this and the other known methods of stimulating germination of these spores.

The early work in this field, adequately reviewed by Miss Ferguson (4), failed to make known any dependable method of germination. Miss Ferguson found that the presence of mycelium or previously germinated spores of the same species served to hasten germination in hanging drops and to increase the number germinating to almost 100 percent. Duggar (2) found that this effect was not produced by sporophore tissue, but only by active mycelium. He obtained 10 percent germination in five days in a few solutions containing various acid phosphates. The reaction of these solutions was not discussed.

Richard and Olga Falck (3) advanced the hypothesis that one or more organic acids, produced by the fungous flora which normally precedes *Agaricus campestris* in outdoor manure beds, were the specific stimulants aiding germination. They obtained stimulation by the use of 0.25 percent succinic acid in malt and manure extracts and distilled water, the germination amounting to 50 percent in nine days. Other acids failed to stimulate germination, although they caused the same pH changes. Bechmann (1), in a series of tests with careful pH control, obtained the best germination, 100 percent in 19 days, at pH values between 5.0 and 6.0. At this reaction, attempts at stimulation with the aid of several enzym preparations and autolyzed tissues, including the tissue of *Agaricus campestris*, failed.

Bechmann considered the sugars in the malt extract used by him to have been the greatest factor in germination. Lambert (7) used a mixture of sucrose, maltose, and dextrose in agar and obtained plentiful germination.

Hein (6) obtained 75 percent germination in 14 days in distilled water alone.

These results lead to uncertainty as to what are the stimuli which cause germination. It is certain that the spores vary in their quickness to respond. Most of the investigators reported the germination of the spores in two groups several days apart. Miss Ferguson's explanation of this phenomenon is that the second group germinates only as a result of mycelial growth from the first. She cited the prompt germination of both these groups in drops containing mycelium from the beginning. The writer has obtained the same results in distilled water in test tubes.

This effect seems to have been obtained by the writer not only from mycelium, but from spores alone when massed. In making the stock cultures referred to above, the spores were in thick masses on the squares of filter paper, and an easily visible mycelium was produced within ten days. When the same approximate number of spores was distributed through the manure, or when the paper carried less spores, the mycelium appeared more slowly and sometimes did not appear at all.

It seems possible that carbon dioxid causes the germination of the more passive group of spores, either directly or through some influence on pH or oxidation-reduction potential. Apparent necessity for carbon dioxid in germination has been reported by Platz, Durrell, and Howe (8) for *Ustilago zaeae*. Germination in a given time increased from 10 to over 50 percent by saturation of the solution with carbon dioxid. Unfortunately this was accompanied by a great change of pH and their cultures were not compared with cultures in which the pH was changed by other means. Rippel and Bortels (9) found that the removal of the carbon dioxid from the atmosphere in which the spores of *Aspergillus niger* were placed increased the time required for germination, and reduced the number germinating. When the few germinated spores produced a mycelium the rest germinated at once, a phenomenon quite like that observed by Miss Ferguson for *Agaricus campestris*. They added the following very interesting statement: "Now if germination did occur here and there over potassium hydroxid at so early a time, this was the case at only those places in which the spores lay together in thick heaps." The parallel between these cultures and those of the writer is striking.

If carbon dioxid is not a factor, some substance such as an acid or enzym, or some effect more difficult to detect, must emanate from the spores and mycelium to cause germination.

NUTRITION EXPERIMENTS

The growth of the mycelium on the plates to which organic materials were added is recorded in table I. The list of these materials was chosen so that it would represent all the classes of organic constituents of undecomposed and decomposing plant tissues as found in manure. Some types of soil organic matter were also included.

The diameter, density, and strand development of the mycelium were recorded after 18 days growth at 25° C. Growth continued in all the vigorous cultures until the plates were full, but the results were recorded at

TABLE 1. *Mycelial Growth of Agaricus campestris After 18 Days at 25° C. Upon Silica Gel Plates With a Variety of Organic Nutrients. Plates Contained NH₄NO₃, 0.05 M, Except Where the Organic Material Contained Available Nitrogen*

| Class | Material | Amount | Diameter (cms.) | Density | Strands |
|-----------------|---|----------|-----------------|------------|------------|
| Acids | Succinic acid | 0.03 M | 3.0 | Sparse | Many |
| | Citric acid | 0.02 M | 0.0 | | |
| Sugars | Arabinose | 0.04 M | 3.5 | Medium | Few |
| | Xylose | 0.04 M | 4.5 | Dense | |
| | Dextrose | 0.04 M | 3.0 | " | |
| | " | 0.12 M | 3.5 | " | |
| | " | 0.20 M | 2.0 | Medium | |
| | Galactose | 0.04 M | 1.5 | Thin | |
| | Maltose | 0.02 M | 3.0 | Medium | |
| | " | 0.10 M | 4.5 | Dense | |
| | " | 0.10 M | 5.5 | " | |
| | Mannite | 0.04 M | 0.0 | " | |
| Starch group | Starch from wheat | 0.25 gm. | 3.0 | Medium | Few |
| | Glycogen | 0.25 gm. | 3.0 | " | " |
| | Inulin | 0.25 gm. | 0.0 | " | " |
| Lecithin | " | 0.25 gm. | 4.0 | Dense | Many |
| Cholesterol | " | 0.25 gm. | 0.0 | " | |
| Gums, pectins | Gum arabic | 0.25 gm. | 1.0 | Thin | Very dense |
| | Peach gum, from wound | 1.00 gm. | 6.5 | Dense | |
| | "Certo," commercial pectin | 2 cc. | 5.0 | Very dense | |
| Hemicelluloses | Xylan | 0.30 gm. | 2.5 | Dense | Few |
| | Agar | 0.55 gm. | 4.0 | Thin | |
| | Coconut, after ether extraction | 0.32 gm. | 2.0 | Dense | |
| | Wheat bran | 0.42 gm. | 5.5 | Very dense | |
| Cellulose | Wheat straw | 0.32 gm. | 4.5 | Dense | Thin |
| | Shredded filter paper | 0.25 gm. | 1.0 | Thin | |
| | Ground cellulose | 0.25 gm. | 2.5 | " | |
| | " | 0.25 gm. | 2.5 | " | |
| Ligno-complexes | <i>Liriodendron</i> wood | 0.25 gm. | 7.0 | Dense | Few |
| | " | 0.36 gm. | 5.0 | " | |
| | German granulated peat moss, commercial "G.P.-M." | 0.20 gm. | 9.0 | Medium | |
| | Wood, cellulose removed by rot, 80 percent lignin | 0.25 gm. | 7.5 | Dense | |
| Humus | 2 percent NH ₄ OH extract of rich soil | 0.25 gm. | 3.0 | Very thin | Few |
| | 2 percent NH ₄ OH extract of rich soil | 0.15 gm. | 1.0 | " " | |
| | "Humus" from New Jersey deposits | 0.20 gm. | 2.5 | Dense | |
| | Nucleic acid from yeast | 0.30 gm. | 4.5 | Thin | |
| Proteins, etc. | Nucleoprotein from yeast | 0.10 gm. | 4.0 | Very thin | Many |
| | Peptone | 0.30 gm. | 3.5 | Dense | |
| | Ghadin | 0.30 gm. | 3.5 | Very dense | |
| | Glutenin | 0.20 gm. | 6.5 | Dense | |
| | Casein | 0.30 gm. | 4.5 | " | |
| | Albumin | 0.30 gm. | 5.0 | " | |
| Blank | No organic material | | 0.0 | | |

18 days because after that time strands began to appear in all the plates. The strands were recorded as "few" when appearing only in the center, and as "many" when extending within $\frac{1}{2}$ cm. of the tips of the hyphae.

The pH of the gel was changed to 4.0 by the addition of succinic and citric acids. As this is far from the optimum pH, the results should be compared only with each other.

It is necessary to note that the materials containing hemicelluloses and lignin were quite impure chemically, and that their classification is only approximate. For example, wheat straw contains cellulose, gums, pectic substances, lignin, and hemicelluloses. Wheat bran was more correctly classified with the hemicelluloses. Woods of the class to which *Liriodendron* belongs contain about 50 percent cellulose, 20 percent pentosans, and 20 percent lignin. The granulated peat moss is much higher in lignin content. The black peaty soil from ancient bogs of New Jersey, sold under the name of "humus," may have been produced mainly from the lignin fraction of the original organic matter, but, like real humus, is quite different from lignin. For the selection and classification of these complex materials the writer has followed the principles of Waksman (12).

The growth of the mycelium upon plates with various nitrogen sources is recorded in table 2. It was necessary in the two series to supply every

TABLE 2. *Mycelial Growth of Agaricus campestris After 18 Days at 25° C. Upon Silica Gel Plates With a Variety of Nitrogen Sources. Plates Contained Dextrose, 0.035 M; Maltose, 0.035 M; and Starch, 0.1 gm., Except Where the Nitrogenous Compound Contained a Carbohydrate Group*

| Class | Material | Amount | Diameter | Density | Strands |
|---------------------|---|----------|----------|------------|---------|
| Salts..... | (NH) ₂ SO ₄ | 0.03 M | 2.5 | Medium | |
| | NH ₄ NO ₃ | 0.05 M | 4.0 | Dense | |
| | NaNO ₃ | 0.03 M | 1.5 | Thin | |
| Urine nitrogen..... | Urea..... | 0.05 M | 1.5 | Dense | |
| | Creatine..... | 0.05 M | 2.5 | " | |
| | Creatinine..... | 0.05 M | 3.0 | " | |
| Amino-acids..... | Glycine..... | 0.05 M | 3.5 | " | |
| | Glutamic acid..... | 0.05 M | 1.5 | Medium | |
| | Arginine carbonate..... | 0.03 M | 2.0 | Very dense | |
| | Leucine..... | 0.03 M | 4.0 | Dense | |
| Proteins, etc..... | Nucleic Acid (Also in table 1)..... | 0.30 gm. | 4.5 | Thin | |
| | Nucleoprotein (Also in table 1)..... | 0.10 gm. | 4.0 | Very thin | |
| | Peptone (Also in table 1)..... | 0.30 gm. | 3.5 | Dense | |
| | Gladiin " " " "..... | 0.30 gm. | 3.5 | Very dense | |
| | Glutenin " " " "..... | 0.20 gm. | 6.5 | Dense | |
| | Casein " " " "..... | 0.30 gm. | 4.5 | " | |
| | Albumin " " " "..... | 0.30 gm. | 5.0 | " | |
| | No nitrogen..... | | 4.0 | Sparse | Many |
| Blank..... | | | | | |

culture with all nutrients except the one to be tested. This was done in the case of the organic series by adding ammonium nitrate whenever the organic nutrient did not contain nitrogen in a form already proved available.

In the nitrogen series, when the nitrogen source did not contain carbohydrate groups, dextrose and maltose were added, each in 0.035 molar concentration, along with about 0.1 gm. of starch. The compounds are grouped in the table according to complexity, with a separate group for nitrogen forms of urine.

The growth was dendritic and the mycelium was irregular in outline in the cultures on sugars. The peach gum and pectin cultures were quite different, spreading evenly and entirely in hyphal form, with many aerial hyphae. The aerial hyphae of the pectin plates presented a powdery appearance, due to great numbers of crystals on their surfaces. The mycelium changed most of the materials classed with the hemicelluloses into semi-liquid pastes. Xylan and fat-free coconut when thus liquefied practically submerged the mycelium, and may have interfered with its growth; the growth was dense, however, in advance of the liquefied zone. The growth on wheat bran, straw, and all the lignin-bearing materials was much the same as the typical dendritic growth on maltose.

The protein cultures resembled the xylan cultures in that the growth was dense, and accompanied by active digestion. The hyphae were straight, pointed directly outward, and were surprisingly equal in length; their tips were observed emerging from the liquefied protein mass unbranched and equidistant, like the teeth of a comb. This type of growth was quite different from the dendritic growth of the maltose cultures, and the enlargement into strands was much slower. Active leading growth in the maltose plates was focused into a few hyphae, and strands were built up from those hyphae after the plates became full. This was not the case in the protein plates.

The growth on nucleic acid and nucleoprotein was extremely thin, with long, unbranched hyphae. This effect was not noted in other plates. The mycelium had a silky appearance, with no strands.

The absence of nitrogen except in the inoculum resulted in the production of many aerial hyphae. The growth was sparse but spread quickly; evidently the nitrogen of the inoculum could be made use of even when spread out quite thinly. The cells of these plates were very commonly bulbous at both ends.

DISCUSSION

The results of these experiments indicate that the organism attacks a great variety of substances. Duggar's conclusion that sugars fail to support it is shown to be erroneous; his culture solution was probably made too concentrated by the addition of sugars. The growth with xylose, dextrose, and maltose supports the opinion that the mycelium grows upon complex organic matter by reason of the production of sugars. It would be interesting to attempt a culture upon cellobiose, which is supposed to be produced by organisms which break down cellulose.

Cellulose supported only a little growth when spread out on silica gel.

There was more opportunity for the products of hydrolysis to diffuse away from the mycelium than in the preliminary paper base cultures, where the paper was packed in tightly. In a recent experiment the writer corroborated the earlier evidence of cellulose breakdown, by measuring the loss in weight of small pieces of filter paper attacked by the mycelium. The loss averaged about 4 percent, and the mycelium was necessarily weighed with the paper. The fibers were swollen and frayed at the ends. In spite of this it seems most likely that the cellulose in a material such as manure is broken down more slowly than some other constituents.

The surprising growth on peach gum and commercial pectin adds two more classes of materials to the list of available foods. The results with the hemicelluloses tested are not very conclusive. The lignin-bearing materials produced the fastest growing cultures of the series. It is important that this mushroom seems to belong with the lignin-destroying organisms, although this must not be considered established by this very limited study. According to Waksman (12) the decomposition of organic matter is usually accompanied by the accumulation of lignin or substances of the same nature, due to the inability of most organisms to attack it. The final residue of such decomposition is of a humus or peaty nature. In support of the theory that *Agaricus campestris* can break down and utilize lignin it may be noted that when the spent manure from mushroom beds is applied to the soil it soon disappears and does not seem to add to the humus of the soil. It would seem that lignin had not accumulated in the mushroom bed.

The growth upon the proteins was not quite typical of the organic series. It was dense and not dendritic. Strands did not appear in most cases until the medium became exhausted. Whether considered from the point of view of organic or of nitrogenous nutrition, it appears that the proteins are very suitable nutrients. From the researches of Waksman (12) we learn that a considerable change of nitrogen from urea and ammonium salts to protein takes place when manure is composted. This process would probably favor the nutritional preferences of the mushroom, and probably takes place in the composting of manure for mushroom growing. The ammonium salts and amino-acids served well as nitrogen sources, as was to be expected, for proteins must be broken down into some of these compounds before the organism can utilize them.

The occurrence of strands in certain plates has no special significance with reference to nutrition, so far as the writer has been able to observe.

The appearance of crystals on the hyphae was noted in all the cultures. More crystals were produced in some than others; the pectin cultures were thickly covered with them. Hein (6) observed crystals which he stated were calcium oxalate. Since more crystals appeared on the aerial hyphae than on the others, and since some of these were soluble in water, it seems possible that some other organic salt or acid was also produced. The pH of the medium is invariably lowered by the spawn in commercial mushroom

production, finally arriving at a value of about 4.5. The same effect was produced in the cultures of Frear (5), with nutrient solutions on a paper base. This pH change may have a profound influence upon the further life of the mycelium; and it may possibly be prevented by chemical means with great benefit to the mycelium. The further study of this matter is being undertaken with hope of useful results.

It should now be possible to make silica gel and also larger scale cultures upon many materials, including composts and fractions prepared from them, and to correlate the results with analytical studies. There should result many improvements in the handling of manures for mushroom production, as well as better understanding of the principles involved. The microbiology of manures will most certainly be found of profound importance. After finding that the organism can obtain nutrients from so many classes of organic compounds, the writer sees no good reason why many other materials can not replace manure in mushroom production.

SUMMARY

1. The mycelium of the "Snow-White" variety of *Agaricus campestris* does not tolerate a medium of nutrient salts and soluble organic substances, in which the total concentration is much above 0.2 *M*.

2. The mycelium is intensely aërobic. In fibrous media it will not tolerate the addition of water if aëration is cut off thereby. Mycelial strands develop profusely in the wetter media, and as their formation necessarily precedes fruiting, a connection between aëration changes, mycelial strands, and fruiting is suggested.

3. The spores of *Agaricus campestris* germinate more quickly in large masses than when separated. The germination stimulus developed in the spore mass appears to be the same as that by which the living mycelium hastens germination.

4. The mycelium was grown on silica gel plates containing materials representative of the main classes of substances in manure and decomposing organic matter. Growth was good with xylose, dextrose, maltose, peach gum, commercial pectin, wheat bran, wheat straw, *Liriodendron* wood, and commercial granulated peat moss; and also on a sample of wood from which nearly all but lignin had been removed by wood-rotting fungi. Several proteins were excellent sources of nitrogen and organic food. Cellulose failed to support vigorous growth. The organism probably can make use of nearly all the substances in manure including lignin. Cellulose, however, would probably not be among the first attacked.

5. The characteristic mycelial growth is dendritic with strand formation. On the proteins the dense hyphal growth is uniform in outline and few strands are produced.

The guidance and assistance of Dr. Rodney H. True throughout the progress of the work is acknowledged with the greatest pleasure. The

writer has also been fortunate in receiving most useful suggestions, as well as samples of some of the nutrients tested, from Dr. Selman A. Waksman.

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STUDIES IN THE ANATOMY AND MORPHOLOGY OF THE COMPOSITE FLOWER II. THE COROLLAS OF THE HELIANTHEAE AND MUTISIEAE

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(Received for publication May 21, 1930)

In Part I of this series of papers¹ a grouping of the types of venation found in the Compositae was given, and the relation of the ray corolla to the disk corolla was discussed. The types of venation were termed the "Aster," "Heliantheae," "Mutisieae," and "Discoid." The "Aster" type usually has four bundles in the expanded corolla, but in some forms there may be five or more. An increase in the number of bundles is brought about by the separation of one of the members of the fused laterals and the subsequent branching of these members. The "Heliantheae" type has eight or more typical veins and two or three other prominent large veins which differ from the first group. The "Bilabiate" type is found in the Mutisieae. The corolla consists of a strap-shaped lobe and two shorter lips which spread laterally. The members of three fused lateral bundles separate at three of the sinuses to supply the ligulate lobe and the two lips. The "Discoid" type includes all forms in which the corolla consists of five fused lateral bundles separating at the sinuses, running along the corresponding lobes, and finally uniting at the apex of each lobe.

The vascular supply to the achene is identical in both ray and disk florets of the "Aster" type. The disk floret has a vascular supply of five fused laterals (pairs of bundles) which persist through the ovary wall and extend into the corolla. In the ray corolla one of the five fused lateral bundles terminates shortly, thus reducing the supply to the corolla to four bundles. The loss of this fifth bundle is observed in certain species of *Aster* and *Senecio*. The split in the corolla of a ray floret is believed to be connected with the abortion or loss of one of the five fused lateral bundles. This provides a line of weakness which is related to the splitting of the corolla. In the Cichorieae and Cynareae the split in the corolla follows the separation of the members of one of the five fused lateral bundles. The ray corolla is considered to be, anatomically, a modification of the disk corolla, and the fundamental structures which are present in the disk corolla are still to be found in the ray corolla.

METHODS

The flowers used in the present study were killed in 70 percent alcohol and thoroughly dehydrated and cleared in Butyl alcohol (26) before em-

¹ Studies in the anatomy and morphology of the Composite flower I. The corolla. Amer. Jour. Bot. 17: 938-952. 1930.

bedding in soft paraffin. Sections were cut 10 microns in thickness, and stained with Crystal Violet and Erythrosin in clove oil (17).

ANATOMY OF THE RAY AND DISK COROLLAS OF THE HELIANTHEAE

The Heliantheae present a striking variation from all other forms in the Compositae in the vascular anatomy of both the achene and the corolla. Before a discussion of the venation in the floret is given, a summary of the gross morphology of the floret will make certain features clearer. *Bidens cernua* L. will serve as a basis for the study.

The achene of the disk floret is four-sided and four-angled. In cross section the achene is quadrilateral with the shorter diameter passing through the anterior-posterior angles. Arising from each angle is an awn which is retrorsely barbed. The lateral awns are longer than the anterior-posterior awns. The disk corolla is virtually enclosed by these awns (Pl. LXI, fig. 19).

The ray floret shows no awns, but the retrorse barbs extend downward along the angles of the achene more prominently on the lateral angles than on the anterior-posterior angles. The under surface of the ray corolla has three strong, appressed veins, which are also clothed with retrorse hairs and are the continuations of the retrorsely barbed angles on the lateral and anterior sides of the achene.

With few exceptions, *Galinsoga* for instance, all genera in the Heliantheae have two or three prominent veins appressed to the under surface of the ray corolla (Pl. LXII, fig. 64). Aside from these veins the venation in the corolla in all of the genera is similar to the venation of a ray corolla of the "Aster" type. The three appressed veins extend to the tips of the lobes of the corolla (fig. 64). In cross section the relation of the appressed veins to the corolla veins is forcibly shown histologically (fig. 63). The appressed veins are surrounded by loose parenchymatous tissue and they are raised upon the basic tissue that forms the corolla. In corollas lacking these structures, as *Aster* and *Helenium*, the vascular bundles lie within the basic tissue of the corolla (fig. 62). The typical veins in the corolla of the Heliantheae occupy the same position within the corolla, and the prominent, large veins stand out in marked contrast (fig. 63). It is clear that these extra veins are not a true part of the corolla, but something accessory.

The problem that now confronts us is the nature of these accessory structures. A study of the nature of the pappus, its occurrence and position in the Heliantheae, will give us a working basis for a theory that offers an explanation for the presence of appressed veins upon the under surface of the ray corolla. The history and bibliography concerning the pappus has been given in full by Small (23) so there is no need to repeat here. Suffice it to say that the question concerning the nature of the pappus is still an open one. Rendle (20) has put the question in concise form by saying:

It seems more in accordance with comparative morphology to regard the pappus as a development of the calyx, but it has also been regarded as representing merely trichomes or emergences. Much as been written as to the true homology of the pappus and the discussion has been summed up by Eichler (1875), who regards it as a modified calyx, and recently by Small (1919), who concludes from its general trichome-structure that it has no relation to a true calyx. Where, as in many *Vernonieae* and *Cynareae*, there are a large number of hairs or bristles arranged in several series of varying size, the less important series may represent merely epigynous outgrowths.

In this study the pappus is considered as an emergence (trichomes, bristles, or scales, etc.) arising from the tips of a gamosepalous structure, which, in its lower part, has become fused to the ovary wall.

Originally there were five sepals, the identity of which is now lost. In some forms, as *Brauneria angustifolia* (DC) Heller and *Marshallia Williamsonii* Small the five scale-like emergences are to be identified as the tips of five sepals. In *Brauneria angustifolia* especially, the fusion of two of the five scales is easily discernible. Most forms, however, show either a reduction in the number of emergences, as the four awns in *Bidens cernua*, or a multiplication, as the trichomes in *Verbesina encelioides* (Cav.) B. & H. An examination of the pappus of the ray florets of the Heliantheae will in the greater number of cases show the presence of emergences in the posterior region of the achene, but not in the anterior region. Where there is a fusing of scales, the scales in the posterior region are in the process of fusion.

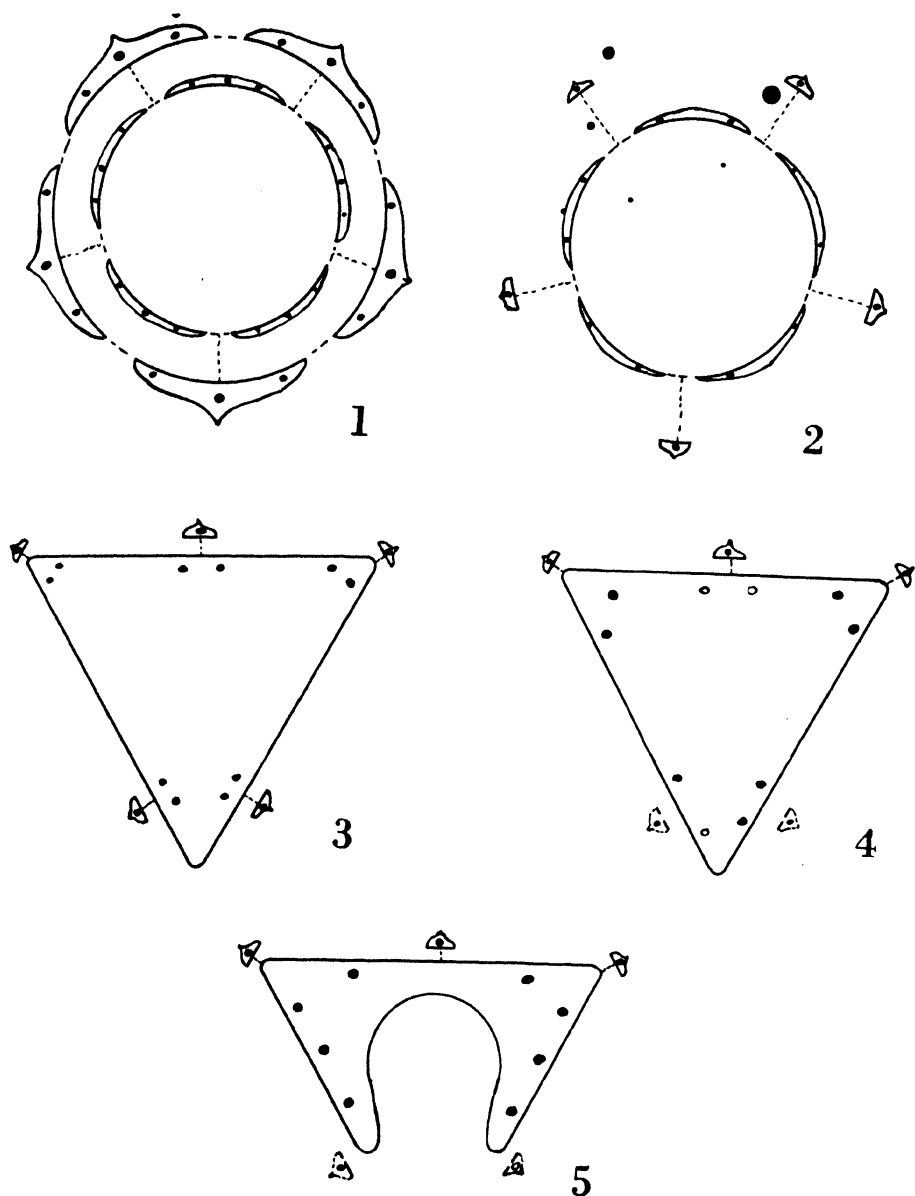
Vascular Anatomy of *Bidens cernua*

A single bundle from the receptacle enters the disk floret. Three branches are given off which may be termed *a*, *b*, and *c* (figs. 1, 2). Branch *c* gives rise to two branches and the remaining vascular tissue supplies the ovule (figs. 3, 4). At the top of the achene each bundle begins to break, and when the bundles reorganize there are five bundles which supply the corolla (figs. 5, 6). To interpret the disorganization of the bundles at the top of the achene as a complete anastomosis is straining the point as much as considering this to be the case in the "Aster" group where such a condition does not exist. When fused bundles supply reduced and altered structures, it is normal for them to break irregularly as they do in the Compositae, especially. Since the contour at the top of the achene is not uniform the bundles must necessarily swing from a position on the periphery of the achene towards the center where the corolla emerges (fig. 6). The two styler bundles are adnate to the corolla bundles and move towards the center taking a position on either side of the styler canal (fig. 7). The calyx crown ("Wulst") breaks into four parts (figs. 8, 9), and in one of the lateral segments there is usually vascular tissue that came from the mass of bundles as they were being reorganized at the top of the achene (fig. 9). At a higher level the stamen bundles free themselves from the corolla bundles as the filaments bend away from the corolla tube (fig. 10). When the corolla lobes are formed the laterals divide at the sinuses and run along the margins of the lobes.

In this form there is no marked difference from the disk corolla of an "Aster" type. The essential points are the reduction in the number of bundles derived from the floral stele and the remnants of calyx bundles being discarded into the base of the awns. Remnants of vascular tissue in the calyx crown are not of rare occurrence in the Heliantheae.

When the supply is given off from the receptacle into the base of a ray floret there is not a mass of fused bundles, but innumerable discrete vascular strands (fig. 11). Some of the lesser bundles are lost, and at the level at which the tissue is massing for the formation of the ovule there are four distinct bundles, one in each angle of the achene (bundles *a*, *b*, *c*, *d*, fig. 12). Since no ovule is formed in the ray floret of *Bidens cernua*, the four bundles extend to the top of the achene where *a* and *b* break irregularly to form the corolla supply (figs. 13, 14). From bundles *a* and *b* are derived the two prominent, appressed bundles of the corolla, *c* continues into the corolla unaltered and is the prominent bundle which lies between *a* and *b* (fig. 17). Bundle *d* divides in the corolla tissue as the size of the corolla increases. A bundle derived from *a* terminates shortly. The split in the corolla (figs. 18, 19) comes at a point in the radius of the corolla which lies over this aborted bundle. This occurrence is comparable to the condition which has been described for the "Aster" type. In *Bidens cernua* the fusion of the bundles is more complete in the base of the floret, so it is difficult to determine the separation of the bundles as the corolla expands. However, the evident abortion of a bundle does suggest that the method of the splitting of the corolla of the Heliantheae is the same as in the "Aster" type.

Originally in the flower of the Compositae there were fifteen corolla bundles and fifteen calyx bundles (text fig. 1). In the corolla the median bundles were lost, the laterals retained and adjacent ones fused (text fig. 2). Upon the assumption that the sepals are modified leaves, a study of the venation in the bracts will indicate what bundle system has probably been retained. The median bundles persist while the laterals in most cases are lost, especially in those bracts that subtend the inner florets of the head. By analogy the median bundles of the sepals have been retained, the laterals either lost or reduced. The vascular bundles represented in the wall of an achene would consist of five calyx median bundles, with possibly vestigial laterals of the calyx supply, and five fused laterals with possibly vestigial dorsals of the corolla supply (text fig. 3). As the floral parts of a floret become more reduced the calyx bundles and corolla bundles would fuse, and in some cases, as in the achene of the Heliantheae, a set of bundles would abort or become fused to another set because of the change in the shape of the achene from a round or ellipsoidal form to a triangular one. The fusion of the calyx or corolla bundles is made possible because of the bundles in each floral whorl that has been retained. The median bundle in the sepal and the lateral bundle of the petal (text fig. 1) lie in the same radius and unite and thus there is formed a bundle that represents the vascular elements of



TEXT FIGS. 1-5. Diagrams to illustrate the relation of the vascular supply of the corolla and the calyx in the Heliantheae. FIG. 1. Each petal and sepal in a floret of Compositae consists of a median bundle and two lateral bundles. FIG. 2. The median bundles of the petals have been lost and the lateral bundles of the sepals. FIGS. 3, 4. Diagrams to show the fusion of the calyx and corolla as the ray floret was modified. Two sepals have been lost, and the vascular supplies of three sepals have become appressed to the under surface of the corolla. FIG. 5. In the expanded corolla the bundles separate to supply the increased size of the corolla.

two floral whorls. The writer believes that in certain species of the Heliantheae with triangular achenes the two posterior sepals have lost their vascular identity, while the three bundles of the anterior sepals have become fused to the ray corolla proper, not losing their identity, even though the fusion in the region below the corolla is so complete (fig. 15). This assumption is supported by anatomical and histological evidence on the basis of the fusion of the vascular supply in the wall of the ovary and the nature of the parenchymatous tissue that surrounds the prominent bundles appressed to the under surface of the corolla.

To represent what has taken place in the Heliantheae of the *Bidens* type a series of diagrams are given (text figs. 1-5). It explains the writer's conception of the phylogenetic development of the ray floret and gives the basis upon which the assumption is made that the vascular system of the calyx has been responsible for the elaborate, heavy veined "corollas" in this group. Text figure 1 shows the venation in the primitive corolla and calyx. Through reduction the midrib bundles of the corolla have been lost and the laterals of the sepals (text fig. 2). In achenes that have become triangular in form (text fig. 3) the two sepals in the posterior region have either disappeared (text fig. 4) or become fused to each other or to one of the lateral sepals in the anterior region. Evidence for this is found in the structure of the calyx crown of *Bidens cernua* and *Helianthus annuus* (fig. 9). As the calyx crown breaks into four segments (fig. 9), a section that occupies a position on one of the lateral angles is considerably larger than the three other segments. This structural feature is found regularly. It is not unlikely that two sepals fused, in the reduction of the vascular elements of the floret from five to four. As the ray corolla broadens the venation consists of the median bundles of three sepals and the fused lateral bundles of either four or five petals. The abortion of a fused lateral bundle accompanies the split in the corolla, thus reducing the number of bundles which supply the corolla to four. The loss of one of the lateral bundles of the corolla that is associated with the dorsal bundle of the sepal occupying the mid-portion in the anterior region of the corolla, also is a factor in the reduction of the bundle system (text fig. 4). In some instances in the ray corolla of *Bidens cernua* these specific laterals in the anterior region are lost, in others, retained. The increase in the size of the corolla of certain species of Heliantheae necessitates an increase in the vascular supply, and therefore the bundles branch freely. This branching condition obscures the relationship that exists between the vascular supply in the tube of the corolla and the expanded ray. Four bundles arising from the floral stele at the base of the achene remain constant in this tribe.

In *Heliopsis helianthoides* (L.) Sweet (fig. 20) the vascular bundles *a* and *b* enter the floret. The bundle *b* breaks into several strands, one of which supplies the ovule. Bundle *a* does not become associated with any of the other bundles and remains intact until the top of the achene is

reached where it divides very freely, and together with the bundles associated with *b* the corolla and pistil supply are formed (fig. 21). The split in the corolla comes at the position of a fused lateral that has aborted (fig. 22). The remaining portions of the two bundles *a* and *b* extend into the ray and become appressed to the under surface of the corolla (fig. 23).

The disk corolla of this species follows the general scheme as shown for the type, the number of bundles reduced to four in the wall of the ovary, and the fifth bundle of the corolla derived from a bundle in the lateral angle of the achene (figs. 24, 25).

It is in the region where the bundle system is disorganized that the supply for the corolla, stamens, and pistil is derived, and in some cases it is impossible to ascertain with any certainty the bundles that are fused. In the case of the split of the corolla the position can be obtained by the number of vascular bundles present at the base of the corolla. Such a count always supports the contention that the split in the corolla is caused by an aborted bundle or the separation of a fused lateral.

There is no fundamental difference in the anatomy of *Helianthus strumosus* L. It is interesting to note, however, that in the disk corolla of this species the midrib bundles are retained.

Rudbeckia triloba L. shows several primitive features that are worthy of note. Even though there are no vestiges of an ovule in the ray floret there are vestiges of stamens (fig. 32). In the disk corolla the stamen bundles are not fused to the corolla bundles but are derived from the bundles at the top of the achene (fig. 34-36), when these bundles separate. In this species the bundles have not fused as completely as in the case of *Bidens*.

Helenium autumnale shows a very simple vascular anatomy. There are no appressed bundles on the under surface of the ray corolla (fig. 62). When the base of the floret is formed from the receptacular tissue there are two bundles present (fig. 27). These bundles divide so that at the level of the ovule there are five bundles in the wall of the ovary and a trace to the ovule (fig. 28). The five bundles persist through the wall of the ovary and extend into the corolla. Just below the split in the corolla one of the bundles aborts (figs. 30, 31). There is no vascular supply to the pistil, although this organ is formed (fig. 31).

The anatomy of *Silphium perfoliatum* and genera similar to this form will be given as a separate study at a later date.

In *Grindelia squarrosa* (fig. 37), a member of the Asterae, the floral anatomy of the floret is strikingly like that which is found in the Heliantheae. The supply to the achene divides into two strands (bundles *a* and *b*), and a trace leads into the ovule (fig. 37). At the top of the achene bundles *a* and *b* separate, forming eight discrete strands (fig. 38). The bundle supply of the pistil consists of one bundle derived from *a* and one from *b*. Between two strands derived from *b* the split in the corolla occurs

(figs. 39, 40). The floral anatomy of the disk floret is similar to the anatomy of the ray floret at the base of the achene. At the top of the ovary the two bundles that have persisted through the ovary wall separate into five bundles which lead into the corolla. The stamen supply is adnate to the corolla bundles.

In this example there has been a greater reduction in the number of strands at the base of the floret than in genera of the Heliantheae that were examined; but the mode of fusing of the bundles, the shape of the achene, and the separation of the bundles at the top of the achene are all points characteristic of the morphology of the Heliantheae. There are no resemblances with the morphology of such forms as *Aster* and *Solidago*, which belong in the same tribe as *Grindelia*.

The disk floret shows no anatomical differences from a disk floret as described for *Bidens cernua*.

"MUTISIEAE" TYPE

The corollas in the Mutisieae are of three kinds: ray, tubular, and bilabiate. The venation of the ray corolla is similar to that of the "Aster" type, four bundles undergoing reduction. At the top of the corolla these bundles join one another, as is common in Compositae; or this connection may be lost, and each bundle is separate. The disk corolla is typical in venation, but in some genera there has been the retention of the median bundles. Tubular flowers, likewise, show this same primitive retention. Neither in Eupatorieae nor Vernonieae, having heads all tubular and alike, was this primitive condition found.

The bilabiate type of flower in Mutisieae repeats again a variation in the division of the fused laterals. The bundle situation in *Trixis angustifolia* DC. does not differ noticeably from the bundle arrangement in the disk corolla of any other composite tribe. The original trace in this species divides into five strands. The dorsal carpellary and anther bundles are adnate to the corolla bundles. When the corolla expands, instead of one fused lateral dividing, as in *Lapsana communis*, three or four of the fused laterals divide (figs. 52, 53). Therefore, in *Trixis angustifolia*, one division of the corolla contains two fused laterals and the halves of the adjacent laterals; the two lobes contain the halves of two laterals (fig. 53).

The head of *Mutisia taraxacifolia* consists of inner bilabiate florets and outer ray florets. The gross morphology of the inner bilabiate floret is similar to the marginal floret of *Trixis angustifolia*. The floral stele divides into five bundles which persist through the wall of the ovary (fig. 41). The supply to the pistil is derived from four of the corolla bundles (figs. 42, 43). At the level of the formation of the stamens the vascular supply to these structures is derived from the corolla bundles (fig. 44). The five fused lateral bundles of the corolla separate (fig. 45) at three sinuses and there then follows the splitting of the corolla: thus there is formed the strap-

shaped lobe and the two lateral lips. The ray floret has the same anatomy as pictured for *Aster laevis* at the base of the floret (figs. 47, 49), but in the expanded corolla there is a greater number of veins (figs. 50, 51). This supply, however, is derived from the separation and branching of the five fused laterals that are so prominent at the base (fig. 47). The abortion of one of the pairs of divided laterals provides the position for the split in the corolla (fig. 51).

DISCUSSION

It has been the general understanding among certain taxonomists that the ray corolla in the Compositae has been derived through that of the Mutisieae by the dropping out of two lobes. Haenlein (12) from embryological evidence maintains this contention. As he says, "In both of these genera (*Leucheria senecioides*, *Moscharia pinnatifida*) the corolla develops regularly and simultaneously with the tubular flowers until the stamens have reached their full development but in most cases the pistil is already distinctly visible. The two inner petals are retarded in their development, while the three outer ones elongate strongly; and in consequence of their unequal lateral growth, they, above the apex of the flower, bend downwards on the inner side. The lateral edges of the lips during these processes meanwhile lie close together; with the unfolding of the flower they bend themselves strongly outward, and the small two-tongued upper lip even rolls distinctly backwards." This does not seem to be sufficient proof for the derivation of the ray corolla from a form like the Mutisieae, neither does it give a basis for his assumption. It merely states a condition, without explanation or reason for the opinion.

As was previously noted, Small attempted, in his study of the corolla of Compositae, to show that the bilabiate corolla was an intermediate step between the tubular and ligulate corollas. Unfortunately, he chose as examples of bilabiate corollas *Calendula officinalis* and *Tussilago Farfara*, species which, taxonomically, are said to have ray florets (22). Therefore, it is not strange that his results were negative, and that his conclusions offer assistance neither in determining the relation of the ray corolla to the disk corolla, nor in explaining the anatomy of the bilabiate corolla as expressed in the Mutisieae.

If the ray corolla of *Aster* had been derived from corollas of the Mutisieae, then the lobing at the tip of the corolla should be an indication of this derivation. An examination of the tip lobing of ray corollas shows no correlation whatsoever with the number of bundles in the ray. In *Chaptalia lyrata* Don. and *Gerbera Anandria* Sch. Bip. there may be no lobing, or there may be one, two, or three lobes. *Rudbeckia hirta* with its many bundles and elaborate anastomosing of the bundles at the tip shows two lobes.

In some Heliantheae, however, three lobes are very prominent, and the calyx bundles associated with the corolla extend to the tips of the lobes (fig. 64). From external evidence the idea that the expanded corolla

represents three lobes of the original corolla may seem plausible, but the origin of the supply in the corolla indicates that the fundamental disk anatomy is represented and that the bundle supply of two lobes is not lacking. In the material studied the abortion of more than one fused lateral or of one bundle in the position of the split in the corolla has never been observed. Von Uexküll-Gyllenband (25) in her general scheme for the phylogenetic development of the corolla shows cases where some corollas in the same head have five lobes, while others have three. This same occurrence has been found in *Rudbeckia triloba* (fig. 64). *Senecio crassulus* A. Gray and *Wedelia buphthalmoides* Griesb. have four lobes (fig. 65). The lobation in *Erigeron canadensis* is of various types suggesting no connection with the bundle system (fig. 61). *Erigeron pulchellus* Michx. is illustrative of this also.

These findings support the contention that the ray corolla was not derived from the disk corolla by the dropping out of two lobes. That two lobes have been reduced in some cases is certain, but the basic vascular anatomy represented in the two lobes is present in the expanded corolla. The elaboration of the lobes has also taken place in some groups. *Centaurea* is the most outstanding example.

As we have seen, the Compositae offer numerous variations in the lobing of the ray corolla, and are not limited to three lobes as would be necessary if the ray corolla had been derived from the Mutisieae type. If we accept the contention of Haenlein, that the ray corolla represents three lobes of the disk corolla, then how can we explain the lobing in *Centaurea*? The marginal tubular florets are elaborate in their lobing. There may be five, six, or seven lobes, and this strongly suggests that in evolution, instead of the reduction in the disk corolla, there has been an enlargement and elaboration of the corolla. The venation in the marginal tubular corolla of the Cynareae consists of five fused lateral bundles that branch freely in the tube of the corolla to supply the additional lobes.

The Mutisieae show as much, or more, variation in corolla form and venation than any other tribe in the Compositae. There are flowers all discoid, flowers radiate, and flowers bilabiate. From this study, it does not seem possible that one type of bilabiate flower gave rise to the multiplicity of forms that we find in the Compositae. It seems far more logical to consider a form like *Perezia*, or *Trixis*, or *Leucheria*, a modification or variation of a disk flower, not a separate type. Furthermore, anatomical evidence bears out the fact that the bilabiate form is a variation of the disk corolla, and in its present state does not suggest a step toward the ray form.

Therefore, cumulative evidence, both macroscopic and microscopic, suggests that the bilabiate corolla of the Mutisieae is not the intermediate form from which the ray corolla has been derived. The evidence is based upon the following characters:

1. The tip-lobing of the ray corolla not being limited to three lobes.
2. The division of the lobes of the corolla, as in *Centaurea*.
3. The variation of the corollas of the Mutisieae.
4. The vascular anatomy of the ray corolla.

AFFINITIES OF THE COMPOSITAE

It had been hoped that a microscopic study of the vascular anatomy of certain families showing gross characters similar to those represented in the Compositae would offer some evidence for the origin of this group. But evidence collected from a study of a five-lobed rubiaceous flower, such as *Pinckneya pubens*, of *Dipsacus sylvestris*, *Goodenia bellidiflora*, *Boopis filifolia*, and *B. anthemoides* was negative. The fusing of bundles as shown in these representatives may be duplicated in any form where reduction of the vascular system is taking place. The closest resemblance to a composite type was found in the Calyceraceae; yet the anatomy of *Boopis anthemoides*, a member of this group, shows how weak even these resemblances are.

At the base of the ovary there are ten bundles which continue through the ovary wall (figs. 54, 55). Five of the bundles at the top of the ovary terminate in masses of sclerenchyma (fig. 56), and when the calyx tips free themselves from the ovary wall the bundles that were held in the sclerenchyma of these structures move inward. From one of the bundles the supply to the ovule is given off (fig. 57). As the ovule in the Calyceraceae is pendulous the trace is not given off until the top of the ovary is reached. In the tube of the corolla the five remaining bundles divide as the supply to the style, stamens, and corolla levels are reached (figs. 58-60). The similarities are few: fusion of lateral and stamen bundles, the retention of the median bundle in the corolla, and connate stamens. All other points are differences.

CONCLUSIONS

From the evidence collected in the study of the composite corolla the writer concludes that there is a fundamental similarity in the anatomy of the ray and disk corollas. There are two main anatomical groups, one the Heliantheae, the other, all forms not included in this tribe. The Heliantheae still show the primitive form or type in the family by the retention of the median corolla bundle. The appression of certain calyx bundles on the under surface of the ray corolla is a marked characteristic of the tribe. The macroscopic venation in the corolla gives the story of reduction in both of the anatomical groups, but it does not show the method by which the bundle arrangement was derived. The abortion or the division of a bundle which is coincident with the splitting of the corolla, determines the bundle arrangement in the ray. The primitive and reduced types of ray florets are evident from the comparative study of the gross venation in the corollas. The position of the tribes taxonomically is no indication of their anatomical rank: Heliantheae are anatomically primitive, but not so taxonomically.

Aster and *Senecio* are far separated taxonomically but from their striking anatomical similarity they are closely related; both are reduced types, their venation showing all stages in the process of reduction. There is no suggestion in either bundle arrangement or tip lobing of a derivation of the ray corolla through the bilabiate form of Mutisieae, but there is sufficient evidence to show that the corollas of the Mutisieae are variations of the disk corolla.

The Compositae, in all respects, are the results of diverse variations from a common ancestry, but the venation does not suggest any evidence of its ancestry. The similarities to other families are those fundamental to all gamopetalous families; no specific differentiations are to be found. The basic flower type in the Compositae, which is represented in the disk floret, has maintained its individuality, despite the many forms that have evolved.

SUMMARY

1. The ray "corolla" of the Heliantheae constitutes a structure which consists of a typical composite corolla plus two or three appressed calyx bundles. The calyx and corolla have apparently become fused, and the vascular supply of these floral whorls united in part. The median bundles of the calyx and the lateral bundles of the corolla are fused and are no longer distinct. In the Heliantheae it is believed that three of the median bundles of the calyx are retained as separate strands and are fused to the under surface of the ray corolla as the distinctly prominent, large bundles. These bundles are surrounded by loose parenchymatous tissue that is distinct from the tissue of the corolla.

2. *Bidens cernua* is used as an example in the Heliantheae to show the presence of these appressed bundles on the dorsal surface of the ray corolla. At the base of the ray floret innumerable discrete vascular strands are given off from a bundle in the receptacle. Four large bundles persist (*a*, *b*, *c*, and *d*). At the top of the achene the four bundles separate to form the corolla supply. The two prominent, large appressed bundles are derived from *a* and *b*, and *c* is the prominent bundle that lies between *a* and *b* in the corolla. Bundles *a*, *b*, and *d* form the typical venation of the corolla. The split in the corolla comes at a point in a radius that lies over an aborted bundle.

3. Four strands given off from the floral stele in the base of the disk floret of *Bidens cernua* furnish the vascular supply of the floret. At the top of the achene one of the four bundles separates into two bundles, thus increasing to five fused lateral bundles the supply that leads into the corolla. This number of fused lateral bundles is always to be found in the disk corolla. The stamen and styler bundles are adnate to the corolla bundles.

4. A diagrammatic scheme which represents the writer's conception of the phylogenetic development of the ray floret is given. It is believed that

the vascular system of the calyx is responsible for the elaborate, heavy veined "corollas" in the Heliantheae.

5. In the ray floret of *Rudbeckia triloba* vestiges of stamens were found. Such a finding emphasizes the relationship that exists between the ray and disk corollas.

6. There are no appressed bundles on the under surface of the ray corolla of *Helenium autumnale*. At the base of the achene of the ray floret there are two bundles. These bundles separate into five bundles and persist through the wall of the ovary, leading into the corolla. One of the bundles aborts, and in a radius in the corolla which lies above the lost bundle the split in the corolla occurs. The anatomy of the disk corolla is similar to the one described for *Bidens cernua*.

7. The morphology of *Grindelia squarrosa* is characteristic of the Heliantheae. There are no resemblances with forms such as *Aster* and *Solidago* which belong in the same tribe as *Grindelia*.

8. The Mutisieae have three kinds of florets: ray, tubular, and bilabiate. The venation of the ray corolla is similar to that of the "Aster" type. The tubular corolla is typical of the venation of the "Discoïd" type. The bilabiate floret is similar to the disk floret at the base of the floret, but in the tube of the corolla the members of three fused lateral bundles separate at the three sinuses to supply a strap-shaped lobe and two shorter lips.

9. The bilabiate corolla of the Mutisieae is believed not to be the intermediate form from which the ray corolla has been derived, as indicated by: 1, the tip-lobing of the ray corolla not being limited to three lobes; 2, division of the lobes of the corolla of *Centaurea*; 3, variation of the corollas of the Mutisieae; 4, vascular anatomy of the ray corolla.

10. No anatomical evidence could be found to show any relationship or connection of the Compositae with any other family usually considered closely related. The closest resemblance to a composite type was found in the Calyceraceae; yet the anatomy of *Boopis anthemoides*, a member of this group, shows only those similarities that are common to gamopetalous corollas in which vascular reduction is taking place.

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DESCRIPTION OF PLATES

PLATE LXI

FIGS. 1-10. *Bidens cernua*. Diagrams of cross section of disk floret. 1, 2. The floral stele that enters the disk floret separates into three bundles (*a*, *b*, *c*). 3. *c* separates forming two bundles, *c*¹ *c*², and the trace to the ovule. 5, 6. At the top of the achene the four bundles

separate. 7. The bundles reorganize and from *b* a bundle has been derived. 8, 9. The calyx crown is discarded. 10. The disk corolla contains five fused lateral bundles which separate at the sinuses.

FIGS. 11–19. *Bidens cernua*. Diagrams of cross sections of ray floret. 11, 12. The vascular supply at the base of the achene consists of four large bundles and several smaller ones which terminate shortly. 13. At the top of the ovary the bundles separate. 14, 15. The vascular system reorganizes. 16, 17. Bundles derived from *a*, *b*, and *c* are large, prominent bundles and are distinguished from the other, more typical bundles.

FIGS. 20–23. *Heliosis helianthoides*. Diagrams of cross sections of ray floret. 20, 21. Bundles *a* and *b* separate to supply corolla. 22. The corolla splits in a radius which lies over an aborted bundle. 23. The corolla has three prominent bundles which differ from the other more typical bundles.

FIGS. 24–26. *Heliosis helianthoides*. Diagrams of cross sections of disk florets. 24, 25. Bundles *a*, *b*, *c*, and *d* supply the corolla and pistil. 26. The stamen bundles are adnate to the corolla bundles.

FIGS. 27–31. *Helenium autumnale*. Diagrams of cross section of ray floret. 27. The floral stele separates into two bundles at the base of the achene. 28. Five bundles are derived from the supply to the floret. 29, 30. Vascular elements are discarded into the calyx crown. 31. The corolla splits in a radius that lies over an aborted bundle.

FIG. 32. *Rudbeckia triloba*. Diagram of cross section of ray floret. Vestiges of stamen structures are present.

FIGS. 33–36. *Rudbeckia triloba*. Diagrams of cross section of disk floret. 33. Four bundles in the wall of the ovary separate to supply the corolla. 34. The stamen supply is not adnate to the corolla bundles. 35. The corolla supply consists of five fused lateral bundles. 36. At the sinuses the fused laterals separate.

PLATE LXII

FIGS. 37–40. *Grindelia squarrosa*. Diagrams of cross sections of ray floret. 37. The supply at the base of the floret consists of two bundles *a* and *b*. 38. Bundles *a* and *b* divide. 39. The supply to the corolla consists of six bundles. 40. The split in the corolla occurs between a fused lateral bundle that has separated.

FIGS. 41–46. *Mutisia taraxifolia*. Diagrams of cross sections of bilabiate floret. 41. Five bundles are derived from the floral stele. 42, 43. The supply to the style is derived from four of the corolla bundles. 44. The stamen supply is adnate to the corolla bundles. 45. The five fused lateral bundles separate. 46. The corolla splits at three points in a radius that lies over four fused lateral bundles that have separated.

FIGS. 47–51. *Mutisia taraxifolia*. Diagrams of cross sections of ray floret in the Mutisieae. 47. The supply to the corolla consists of five bundles. 48. The supply to the pistil is derived from two of the corolla bundles. 49. The calyx crown is discarded. 50. The bundles in the tube of the corolla separate and branch. 51. The split in the corolla occurs in a position in a radius that lies over an aborted bundle.

FIGS. 52–53. *Trixis angustifolia*. Diagrams of cross sections of bilabiate floret with two lateral lips. 52. The bundles in the tube of the corolla separate. 53. The corolla splits in a radius that lies over three fused lateral bundles that have separated.

FIGS. 54–60. *Boopis anthemoides*. 54. The supply at the base of the achene consists of ten bundles. 55. At a slightly higher level in the ovary wall the ten bundles are embedded in masses of sclerenchyma. 56. At the top of the ovary five of the bundles terminate. The calyx tips are discarded. They do not contain vascular bundles. 57. The trace to the ovule is given off from one of the bundles that leads into the corolla. 58. In the tube of the corolla the bundles divide. The supply to the style is derived from two of the corolla bundles. 59. The stamen bundles are adnate to the fused lateral bundles of the corolla. 60. The corolla consists of five dorsal bundles and five fused lateral bundles that separate at the sinuses.

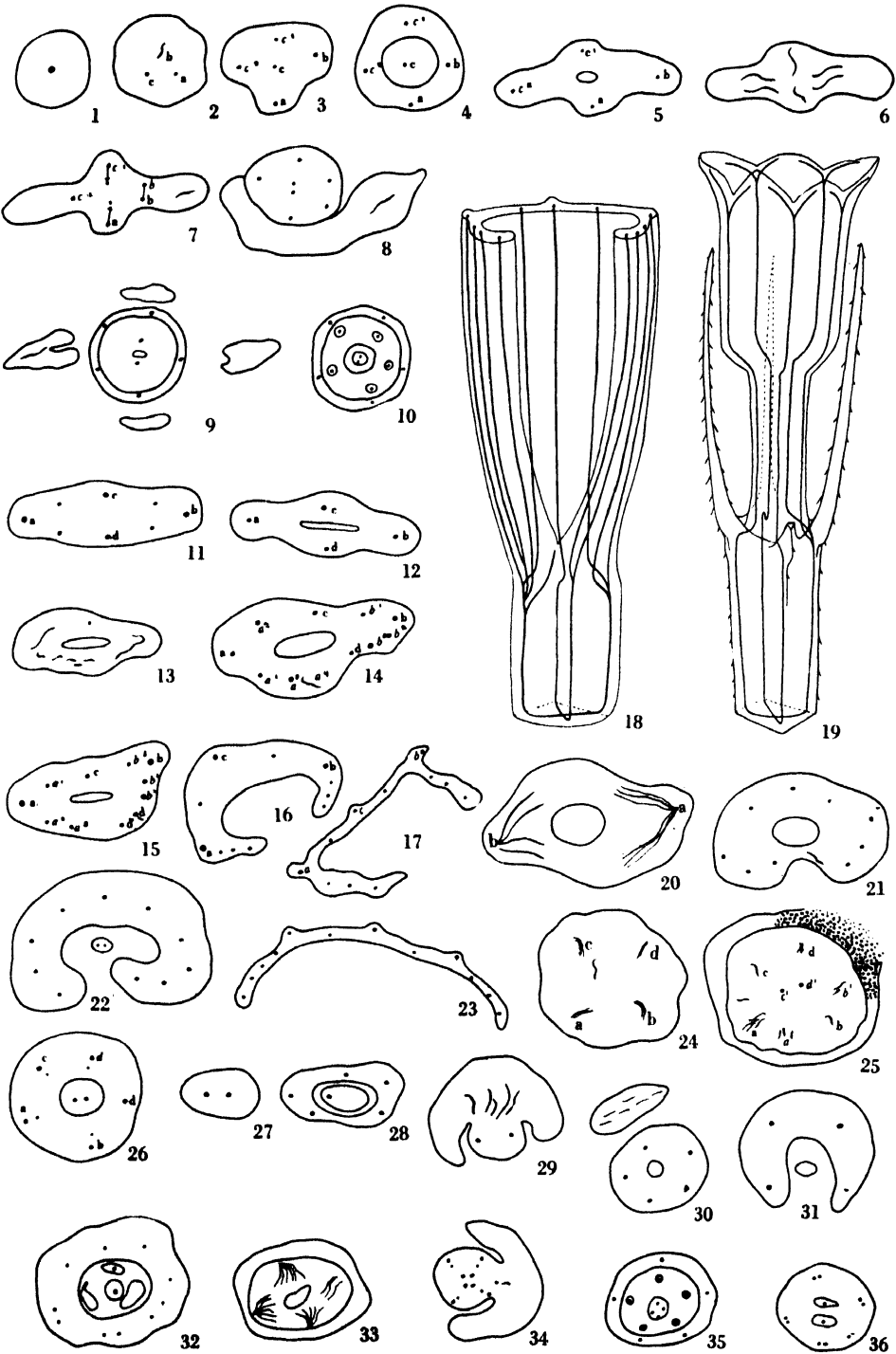
FIG. 61. *Erigeron canadensis*. Diagram of tip lobing in relation to venation.

FIG. 62. *Helenium autumnale*. Diagram of cross section of corolla to show position of vascular bundles in corolla.

FIG. 63. *Bidens cernua*. Diagram of cross section of corolla to show the prominent, large, appressed bundles and their relation to the typical venation.

FIG. 64. *Rudbeckia triloba*. Diagram of ray corolla with five lobes to show relation of tip lobing to venation.

FIG. 65. *Senecio crassulus*. Diagram of ray corolla with four lobes to show relation of tip lobing to venation.



KOCH: COMPOSITE FLOWER



KOCH: COMPOSITE FLOWER

INTRAMOLECULAR RESPIRATION OF TOMATO FRUITS¹

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These experiments on intramolecular respiration have been conducted as a part of a general study of the development of tomato fruits. Nitrogen and in two experiments hydrogen have been used to replace air. These gases were first passed through a wash bottle containing NaOH which absorbed the CO₂ and then through three bottles of chromous chlorid which absorbed any oxygen that was in the gas. The CO₂- and O₂-free gas was then passed through the respiration chamber by the pressure in the tank and reduction of the pressure in the system by suction. As it came from the chromous chlorid the gas was frequently tested for oxygen by passing it over phosphorus, always with negative results. The respiration chamber was always kept at atmospheric pressure and was immersed in a constant temperature bath held at 25° C. A control in air otherwise similarly treated was run simultaneously. The fruits were usually picked during the afternoon and put into the chamber through which air was drawn over-night in order to establish an equilibrium, before any collection for analysis was made.

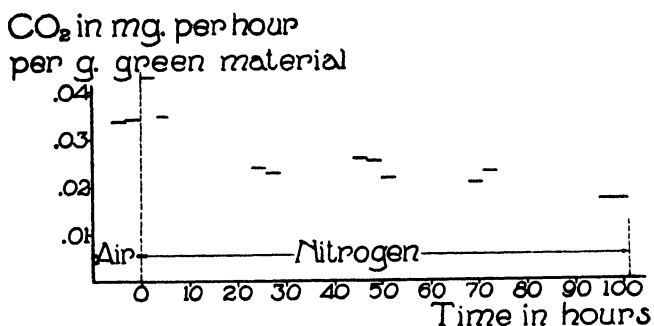
The CO₂ was collected in 0.1 M Ba(OH)₂ contained in modified Pettenkofer tubes, and titrated with 0.1 M oxalic acid. Further detail may be obtained from a previous paper (5).² In the present experiments the Pettenkofer tubes had a diameter of only 9 mm. in the main portion, with both ends enlarged somewhat, to facilitate the insertion of stoppers. The use of tubes with a small diameter made it possible to utilize a volume of only sixty cubic centimeters of Ba(OH)₂ and yet the gas passed through a column of liquid long enough to absorb the CO₂ completely. With such a small volume of liquid the time of the individual collections could be shortened to one hour in some instances. In all but one experiment the length of time for collection of each sample was from one to three hours. Such short periods enabled one to follow more closely the changes in the rate of CO₂ production. In the early experiments three hour periods were used almost exclusively, but in the later experiments several fruits were used and the time could be shortened to one hour when the gas production was at its maximum.

After some preliminary trials an experiment with a ripe fruit weighing

¹ Paper from the Department of Botany of the University of Michigan, No. 341.

² No reference has been made here to the rather extensive literature on anaerobic respiration of flowering plants, since previous experiments have been so very different from the ones here discussed that it has seemed unnecessary.

78.9 g. was started in the afternoon and air drawn through the apparatus without any collection of gas until the next morning. This experiment is the only one conducted in which there was no control in air. The collection in air was made for six hours before nitrogen was introduced. As shown in text figure 1³ there was an immediate rise of CO₂ production when nitrogen



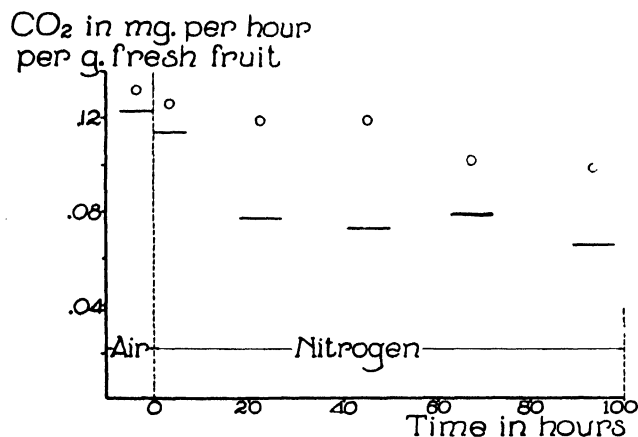
TEXT FIG. 1. Respiration of a red-ripe tomato fruit. In this as well as in all other figures the length of the lines denotes the duration of a collection. Sometimes two collections may have the same value and the two together will be represented by a line double in length, as the last two collections in this experiment. At the zero point air was replaced by nitrogen.

was introduced, amounting to 28 percent, but during the second period of collection the CO₂ production was back to the original level. During the night nitrogen was run through the apparatus at a rate similar to that during the daytime when collections were made; if, as it sometimes happened in the early experiments, the gas flow had slowed down somewhat during the night, no collections were made for several hours after the flow had been corrected in order to wash out thoroughly any CO₂ that might have accumulated in the container or in the tissue. In this experiment the gas flow was less in the morning than during the preceding day and four hours were allowed to elapse before any collection was made. During this time the gas flow was the same as the preceding day. The rate of gas flow was determined by the distance between bubbles in the Pettenkofer tubes. The size of the bubbles was usually the same. This method is not very exact, but it was noted that if the gas flow thus measured was nearly the same, there was no difference in the amount of CO₂ collected. By referring again to the graph in text figure 1 it will be noted that 24 hours after the nitrogen was introduced the CO₂ production was 71 percent of that at the beginning. The experiment was continued for 102 hours in nitrogen. During this time there was a gradual decrease in CO₂ production, which fell to 50 percent

³ In all of these experiments the results for the experimental fruits in nitrogen are represented by either solid or interrupted lines, one line for each collection and the length representing the duration. The control is represented by circles, and each circle is the average of the several collections made each day. For the sake of simplicity the experiment number will be the same as the number of the figure representing it.

at the end. As will be seen from other graphs in which ripe fruits were also used the control shows a decrease in production of CO_2 during an experiment. During the first 24 hours in nitrogen there is then a decrease of 29 percent in the CO_2 production, while during the remaining 78 hours there is a decrease of only 21 percent. In the same length of time a control fruit would have shown nearly the same decrease.

After the experiment on the ripe fruit others were conducted in the same manner in which fruits of various degrees of ripeness and green fruits growing at various rates were used. Of these only three experiments with their controls need to be mentioned, because the others conform to type.

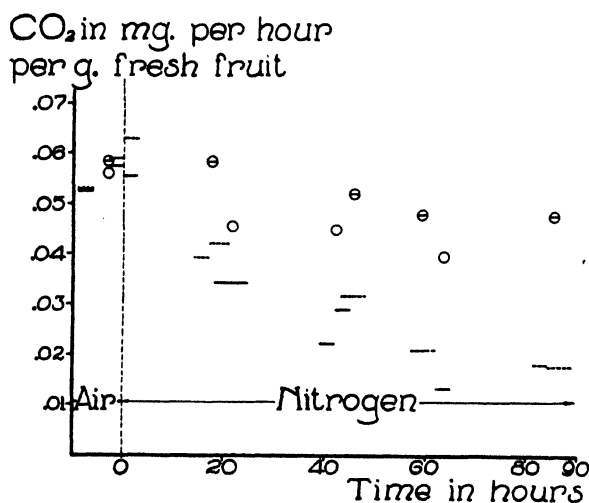


TEXT FIG. 2. Respiration of a green fruit, growth rate 50 percent per day. The circles represent the control and in all experiments except this one each circle is the average of the several collections for the day.

Text figure 2 illustrates the respiration of a green fruit and its control. The fruit weighed only 4.2 g. and had a diameter of 2.11 cm., and had had a growth rate of 50 percent per day. The control weighed 4.56 g., was 2.08 cm. in diameter, and had been increasing at the rate of 45 percent per day. Due to the small size of the fruits the collection period had to be very long, seven hours the first day and nine hours the following days. When nitrogen was introduced there was no apparent increase in CO_2 . Whether there was an initial increase which was followed by a greater decrease producing an average lower than at the beginning, or whether there was a slow decrease from the beginning cannot be stated. From subsequent experiments in which several fruits were used and the periods of collection were short it would seem that there is an initial rise followed by a drop, so that the average for seven hours would be less than normal. A day after the nitrogen was introduced the respiration had decreased to 63 percent of the original, while the control was 92 percent of its original value. During the next two days the rate of CO_2 production remained nearly constant in the experimental fruit, but on the fourth day there was a considerable decrease and at the end of

95 hours in nitrogen the respiration was 54 percent of the original, whereas in the control it was still 74 percent of its original rate. The gradual decrease in the CO_2 production of the control is very typical. There was hardly ever a large decrease during any one day, but at the end of the experiment it too had dropped considerably below the initial rate. It is to be noted that these green fruits produced more than three times as much CO_2 as the ripe fruits represented in text figure 1.

Several experiments were carried out in which faintly pink or yellow fruits were used but only two of them are presented in text figure 3. The



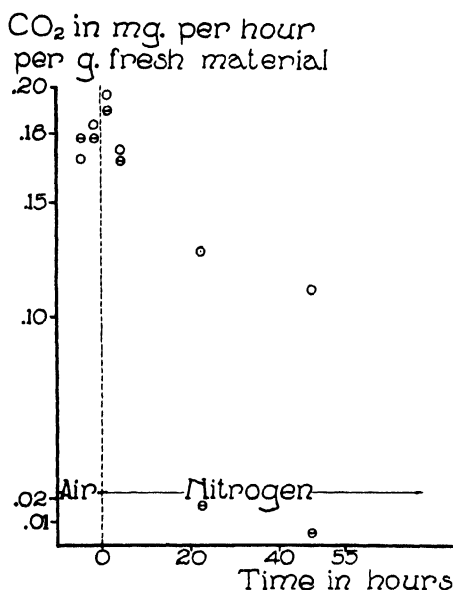
TEXT FIG. 3. This figure illustrates two experiments with their controls. The fruits were pink. The dotted lines and the circles with a line through them constitute one experiment and the solid lines and circles the other experiment.

point of note in these experiments is that the CO_2 production of the fruits in the nitrogen decreased rapidly from the first day. At the end of 65 hours the CO_2 production of one was only 24 percent of its initial output, while its control was still 73 percent. The other experiment produced 32 percent after having been 85 hours in nitrogen and its control 78 percent. These pink fruits behaved normally when in air, but very differently from the others when in nitrogen. They never deviated from this performance.

To find out whether the leaves reacted as the fruits did, one experiment was conducted with them (text fig. 4). The circles with the lines through them represent the leaves in nitrogen. The leaves did not hold up even as long as the pink fruits. After 50 hours in nitrogen the leaves produced only 3 percent as much CO_2 as they did at the beginning of the experiment; the leaves in air respired 64 percent as much as at the beginning.

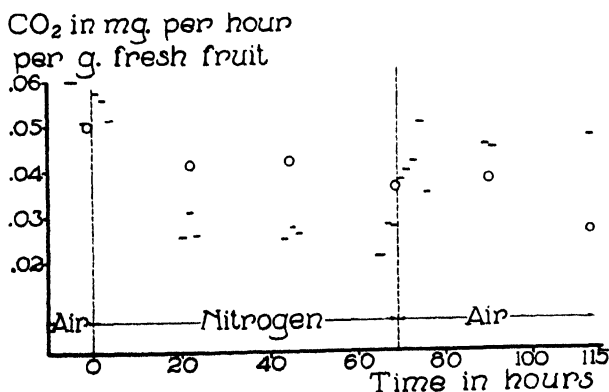
These experiments, which are well illustrated by the four given here, all show that the production of CO_2 is well sustained in green or ripe fruits in the absence of oxygen but this is not true of the leaves or the pink fruits.

The fact that in absence of oxygen tomato fruits produced a considerable amount of CO_2 suggested the importance of determining what would happen if air was again introduced. Several experiments were performed in which air was reintroduced after the fruits had been some time in nitrogen and of these, two are chosen as typical.



TEXT FIG. 4. An experiment with tomato leaves. The circles with the lines denote the readings of the leaves deprived of air.

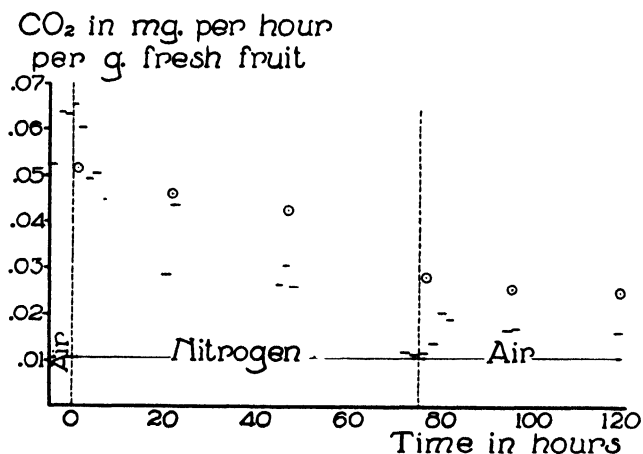
Text figure 5 represents an experiment in which were used two ripe fruits with a total weight of 151.5 g. and diameters of 5.28 and 5.72 cm. Two slightly larger fruits were used for the control. The three readings taken before the nitrogen was introduced were very irregular, but after that the



TEXT FIG. 5. Respiration of a red-ripe fruit and its control. After 69 hours in nitrogen air was reintroduced.

CO₂ production was quite uniform. The fruits were deprived of air for 69 hours and at the end of this period the CO₂ production had decreased to 49 percent of the initial. As soon as air was reintroduced there was an immediate increase in CO₂ output and within six hours it had reached 102 percent of the original or had increased 104 percent over what it was in nitrogen. This rate of respiration continued during two days, when the experiment was discontinued.

The second experiment of this group was one in which two pink fruits were used. Their diameters were 5.16 and 5.92 cm. and their combined weights 150.5 g. The control also had two fruits, slightly smaller. Text



TEXT FIG. 6. Respiration of a slightly pink fruit. Air reintroduced after 75 hours in nitrogen.

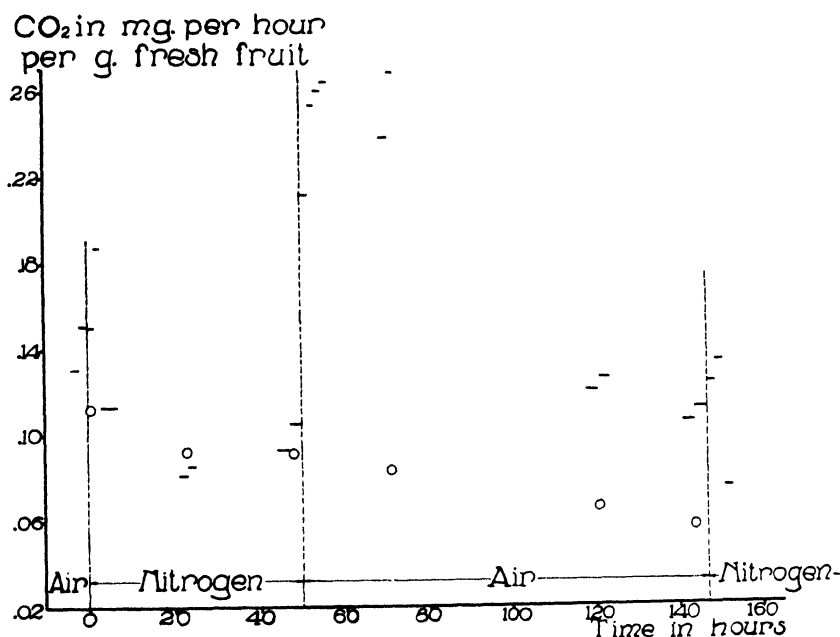
figure 6 illustrates this experiment. As usual with the pink fruits the respiration fell rapidly and after 75 hours in nitrogen the CO₂ output was only 19 percent of the initial. When air was reintroduced there was here also an increase in respiration, to 35 percent that of the initial or 80 percent over the rate in nitrogen before the air was introduced. During the two following days the rate of respiration remained nearly stationary. At the end of the experiment, 120 hours after the nitrogen was introduced, the control respired 49 percent of the original and the nitrogen treated fruits 27 percent.

Both of these examples show that when air is reintroduced there is an increase in respiration. This increase was sometimes, though not always, to a level considerably above the original.

Another group of experiments was initiated in which nitrogen was introduced a second time and in two experiments the air was introduced after the second nitrogen period. Three experiments from this group will be given, not because they are so very different in response, but since they represent fruits in very different stages of development.

The first of these was concerned with eight green fruits having an average diameter of 2.15 cm., a combined weight of 35.1 g. and a growth of

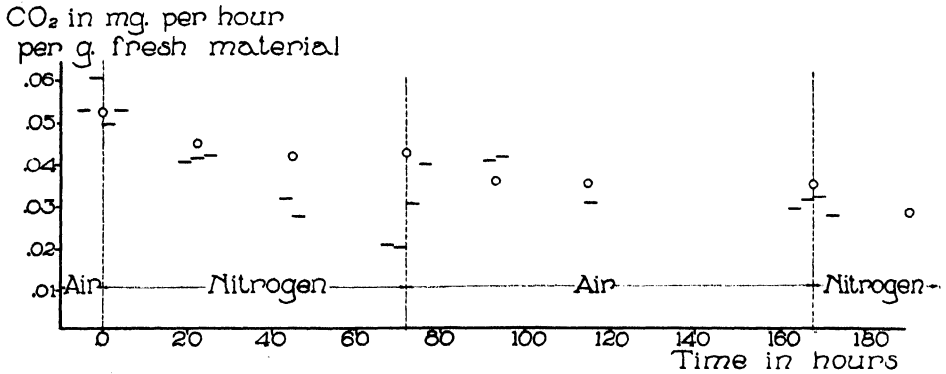
13 percent per day (text fig. 7). The control consisted of seven fruits of about the same size, also showing a growth of 13 percent per day. When the nitrogen was introduced there was a very decided increase in CO_2 production during the second period, which was followed by two periods of very much lower production. After a period of 50 hours in nitrogen air was introduced and there was an immediate rise in respiration which continued



TEXT FIG. 7. Respiration of eight green fruits, average diameter 2.15 cm., growth 13 percent per day, and their control. Air was reintroduced after 50 hours in nitrogen, and after 97 hours in air nitrogen was introduced a second time.

for the next three periods until the rate of CO_2 production was 189 percent of the original or an actual increase of 166 percent over the rate in nitrogen just before air was introduced. The average of two determinations made the next day was a little lower. The experiment was continued in air over Sunday and by Monday a considerable drop had occurred but the rate was still very much above that of the control. After 97 hours in air, nitrogen was introduced a second time and this time there was also a rise in respiration which lasted for four hours; but during the third period there was a very definite drop, to considerably below the rate in air immediately preceding the second nitrogen introduction. In this experiment, which lasted 153 hours after the nitrogen was first introduced and in which the fruits were, all told, 56 hours in the nitrogen, the CO_2 production at the end was greater than in the control which had been in the air during the whole period. The respiration of the control had dropped to 51 percent when the experiment was ended.

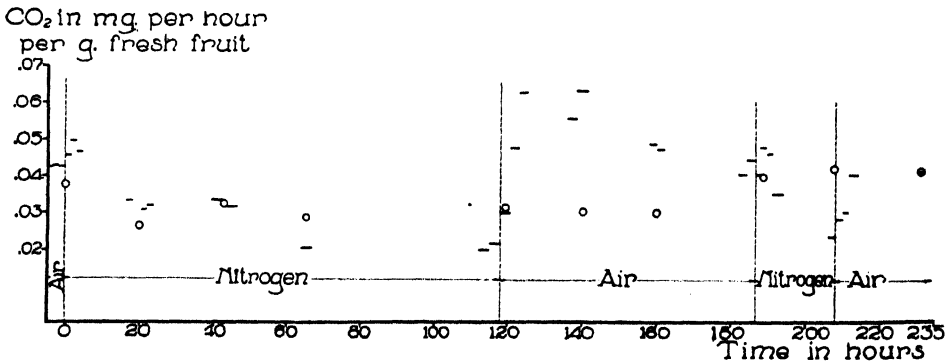
In a second similar experiment (text fig. 8) one fruit, yellow-orange with green near the stem, weighing 94.86 g. was used. The control had the same color and weighed 85.58 g. These fruits were 42 hours in the respiration chamber with air drawn through before any gas was collected. At neither time of nitrogen introduction was there a rise in respiration. It



TEXT FIG. 8. Respiration of a yellow-orange-green fruit, and control.

will be noted that in nitrogen the respiration decreased quite rapidly but not so much as in experiments with fruits that were pink or slightly yellow, yet more than with fruits that were orange or red. On the introduction of air there was a gradual rise to the 74 percent level and this was maintained during the following day. This was an increase of 108 percent over the rate in nitrogen, when air was again admitted to the respiration chamber.

The third experiment (text fig. 9) of this group was rather long. It



TEXT FIG. 9. Respiration of two green fruits, growth 1.7 percent per day. In this experiment the experimental fruits were in nitrogen and air alternately for 232 hours. The control shows an increase and this is interpreted as being due to ripening. At the beginning of the experiment these fruits were green, while at the end they were yellow-orange.

lasted 11 days or 235 hours from the time nitrogen was first introduced through the last collection when the fruits were in air. Two green fruits were used with a total weight of 120 g. and a daily growth increment of 1.7

percent. The control also consisted of two green fruits weighing 130 g. with a growth increment of 1.7 percent. When air was first displaced by nitrogen there was a rise in CO_2 production, which was not very great. During the 118 hours in nitrogen there was a gradual decline in CO_2 production from 76 percent of the original, 20 hours after the start, to 48 percent at the end. When the nitrogen was replaced by air after this long period of intramolecular respiration the CO_2 production rose in seven hours to 147 percent of the original or an increase above the respiration in nitrogen of 206 percent. This rate continued the next day, but fell on the third day, reaching 99 percent on the fourth. When nitrogen was introduced a second time there was a rise, followed by a very sharp decline to 54 percent. A second replacement of nitrogen by air brought production of CO_2 up to 110 percent, at which level it remained during the next day. This experiment is interesting because of the long sustained intramolecular respiration and the very large increase noted both times when the air was reintroduced. The experiment is also of interest because of the behavior of the control, which reached its lowest respiration level (67 percent) during the second day of measurement, after which there was a slight increase that was maintained for several days until on the ninth day there was a marked increase which continued the next day. The results fall in line with those presented in a previous paper (4) indicating that tomato fruits reach their minimum rate of respiration at about the time they begin to change color, after which time there is an increase up to the time they become orange-red. At the end of this experiment the fruit in the control was yellow-orange, while at the commencement it had been green.

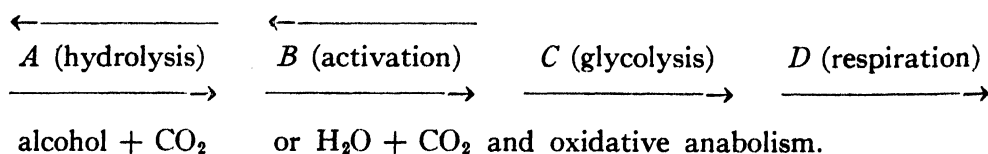
Before discussing the experiments it may be appropriate to summarize the results briefly. In most experiments there was an increase in CO_2 output when nitrogen replaced air either the first or second time. This increase was quickly followed by a very large decrease, after which there was only a very gradual fall, except in pink or yellow fruits, in which the decrease was quite large during the whole of the nitrogen period. The replacement of nitrogen by air always brought about an increase in CO_2 output, varying from about 80 percent to over 200 percent above that in nitrogen just before air was reintroduced. In some experiments much more CO_2 was given off in air than during the periods before nitrogen was introduced, whereas in others there was less, but in only one experiment was there less than in the control at that time. Sometimes several periods were required before the maximum CO_2 output had been reached, but in all instances the respiration during the first period in air was higher than the last period in nitrogen. After air was reintroduced the CO_2 production remained high for the remainder of the aerobic respiration.

A discussion of these experiments can be divided into two sections, one dealing with the intramolecular or nitrogen phase and the other with the aerobic or oxygen phase.

In most of the experiments and in all in which the time of collection was short, there was an initial rise in CO_2 output when nitrogen replaced the air. How is this increase to be explained? In the absence of oxygen, theoretically only one-third of the carbon is used in CO_2 formation while in air all of the carbon goes into the formation of CO_2 , so that if the production of CO_2 were the same in both, three times as much carbon material would be used in intramolecular respiration as in aerobic respiration. In most experiments, however, there is an increase in CO_2 production when O_2 is excluded.

One explanation that comes to mind is that this increase is only apparent, and results only from an outpouring of CO_2 already in the fruit in the intercellular spaces, due to the difference in diffusion of oxygen and nitrogen. If nitrogen diffused more rapidly than the oxygen the total pressure in the interior would increase and some CO_2 might be driven out. However, their diffusion rate is practically the same. The coefficient of diffusion of nitrogen into pure water at 19°C . is $1.9 \times 10^5 \text{ cm.}^2/\text{sec.}$, and that of oxygen at 18°C . is $1.9 \times 10^5 \text{ cm.}^2/\text{sec.}$ (6). Of course there might be some difference in their ability to penetrate the membranes in the fruit, but apparently in the case of nitrogen and oxygen there is no such difference. Hydrogen, on the other hand, does seem to bring about an outpouring of CO_2 . In two experiments to be discussed later hydrogen was used to displace air and here the initial rise of CO_2 output when air was replaced was greater than when nitrogen was used as a substitute. This was probably due to the more rapid diffusion of hydrogen, which increased the gas pressure within the fruit. Flow of the mixture of gases within the fruit to the outside, through the stem scar, would take place until equilibrium was attained. This view is further strengthened by the fact that when hydrogen was replaced by air the increase was less than when nitrogen was used. Here the hydrogen diffused out more rapidly than air came in and some CO_2 remained in the intercellular spaces because of a reduction in total pressure. Hydrogen has, of course, a much greater rate of diffusion than nitrogen, and it seems that the increase in CO_2 output on replacement of air by nitrogen is not due to nitrogen driving the CO_2 out of the system, but rather to an actual increase in CO_2 production.

How is this increase to be explained? A schema proposed by Blackman (1) may supply an explanation, as follows:



(A) he considers as any complex carbohydrates which are hydrolysed to any number of sugars (B); these sugars are changed to the more unstable ring compounds (C) in activation; the active sugars are changed to the three

carbon atom compounds (*D*) and this is called glycolysis; the compounds (*D*) are then used in respiration, forming CO_2 and H_2O in air and CO_2 and alcohol in absence of air. Blackman assumes that during aërobic respiration a large part of the intermediate products (*D*) are not used in respiration but go back into the system. When air is excluded, however, this process stops, leaving more material to be used in respiration. This intermediate material accounts for the increase in CO_2 production on introduction of nitrogen and as soon as this excess is used up there is a drop to a lower level characteristic of intramolecular respiration.

If all of the product *D* is used in respiration in absence of air why does the rate of CO_2 production decrease after the first few hours? Blackman assumes that the production of *D* is not influenced by oxygen, but that the formation of either *B* or *C* or possibly both is influenced by the oxygen pressure and that in absence of oxygen they decrease, and even though all of *D* is used in respiration, the total is less than in air. This explanation is not satisfactory, but the writer has none of his own to offer. When hydrogen replaces air part of the increase is only apparent and is due to a more rapid penetration of hydrogen, which then drives some CO_2 out of the system because of the increased pressure in the tissue, but here also there is an actual increase in respiration as explained above.

With the exception of those experiments in which either pale yellow or pink fruits were used, the rate of CO_2 production after the first 24 hours in nitrogen decreased only slightly, not any more than that of similar fruits in air. This would seem to show that respiration in nitrogen is a well balanced or regulated process which merely produces less CO_2 than when the fruits are in air. It is on a lower CO_2 -producing level than in air.

When air replaced nitrogen there was always an increase in CO_2 production. In some experiments this increase amounted to over 200 percent, although in others it was less than 100 percent. If the introduction of nitrogen does not bring about an outpouring of CO_2 from the tissue, neither can its replacement be accredited with this, and an explanation for the increase must be looked for in another direction.

It was first suggested by Pfeffer and later by other investigators that in the absence of air substances are produced which are rapidly consumed in the air to produce CO_2 . These are not the intermediate compounds (*D*) of Blackman, but probably a decomposition product of them. In intramolecular respiration only one-third of the carbon appears as CO_2 . The other two-thirds are supposed to be used in the formation of alcohol, but not much alcohol is found in higher plants when they have been in nitrogen. So far in these experiments no careful attempts have been made to detect alcohol in tomatoes. However, in the crude qualitative tests made there has been no definite detection of alcohol. If alcohol is not formed other products may be, which are later used in aërobic respiration. In many plants, particularly in succulents, quantities of organic acids are found.

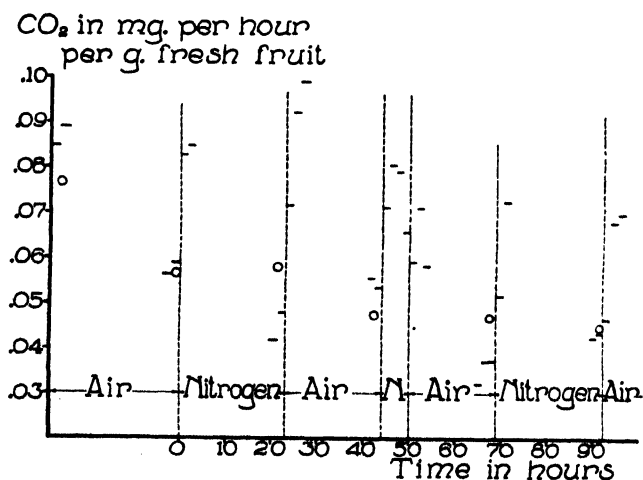
Any number of compounds might be formed in the absence of air which are easily oxidized in the presence of oxygen.

Meyerhof (7) has a somewhat different idea about the increase in respiration after a period of intramolecular respiration. It has been shown that the lactic acid formed in the contraction of muscles disappears during the recovery or aërobic phase of muscular activity. He thinks that the lactate ion stimulates aërobic respiration, which utilizes not the lactic acid but the glycogen, and that part of the energy so obtained is used to convert the lactic acid formed during the intramolecular phase of muscular activity back to glycogen. The stimulation of aerobic respiration of a muscle by the lactate ion can also be demonstrated by placing an uninjured muscle in a lactate-containing salt solution, when the respiration will be increased two to three times.

Here are two different views attempting to account for the increase in respiration which is noticed when intramolecularly respiring organisms are brought back into air. Both hold that a compound or compounds are formed during the intramolecular period which are responsible for this increase, but according to one these substances are actually used in producing the increased amount of CO_2 , while according to the other they act as a stimulant.

If something is formed in these tomato experiments during the nitrogen period which is later used in aërobic respiration, shorter periods of respiration in nitrogen should produce less of this material than longer periods, and respiration in air should increase less, or at least the period of increase should be shorter, than when the nitrogen periods are long, unless so much of this hypothetical intermediate substance is formed as to become poisonous.

To investigate this idea several experiments were conducted in which

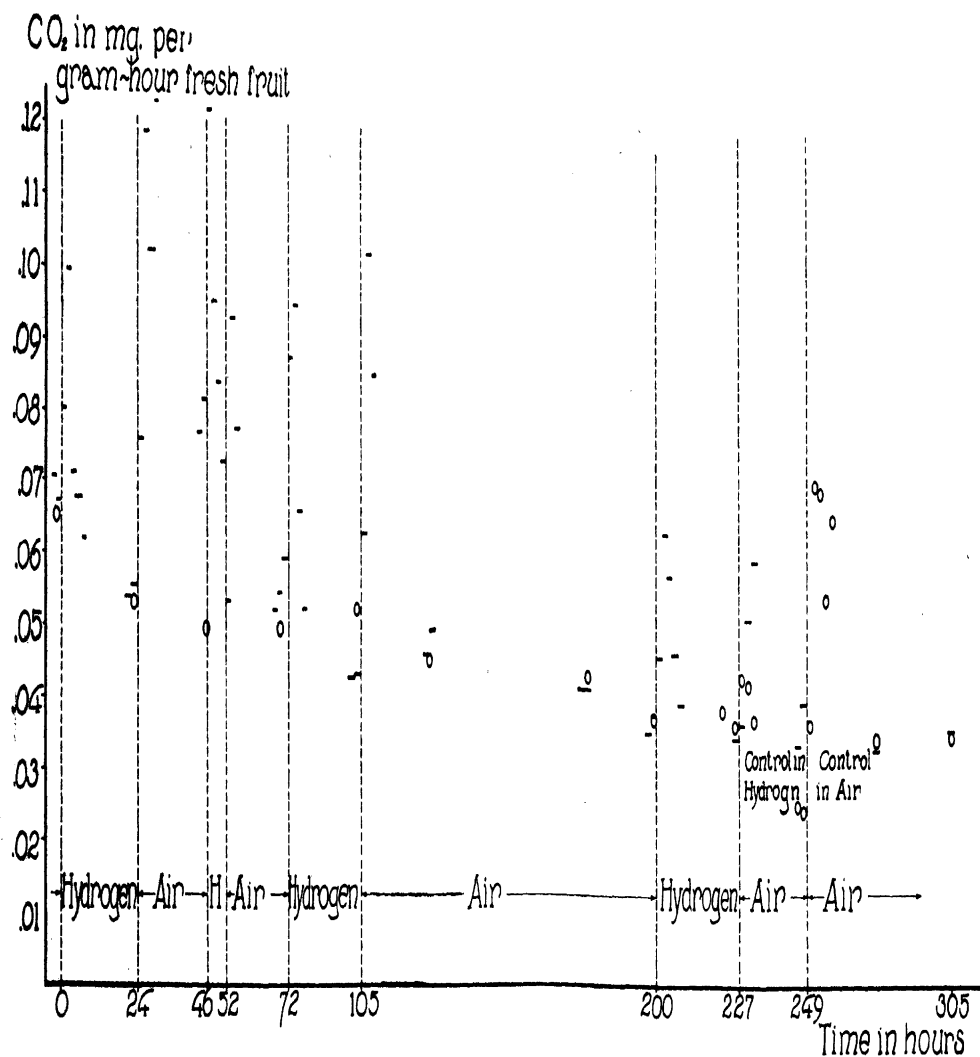


TEXT FIG. 10. Respiration of six green fruits, growth 6.3 percent per day. This experiment was conducted to determine the effect of the length of the nitrogen period on the amount of CO_2 produced when air was again admitted. It shows that following a short nitrogen period the CO_2 is much less than that following a longer nitrogen period.

the length of the nitrogen periods was varied. One of these is given here. In experiment 10 figured in text figure 10, six green fruits having a total weight of 118.25 g. with an average diameter of 3.4 cm. and a growth rate of 6.3 percent per day were used. The control fruits were slightly smaller and had a growth rate of 5.84 percent. This experiment was started in the evening and the first determinations made the next morning. It was continued in air for this day and part of the next. It will be noticed in the graph that both the experimental fruits and the controls decreased much in their respiratory rate during these first 24 hours. When nitrogen was introduced there was a greater increase in CO_2 than usual. Next day the CO_2 production was down to 77 percent of what it was at the time nitrogen was introduced. This result is typical of fruits growing at the rate these were. The increase on nitrogen replacement was about as great as could be expected, but the fall to 94 percent in 20 hours was greater than usual. Next, a nitrogen period of only six hours was used and this time the rise in air was very slight and was followed by a decrease within a few hours and the next morning it was down to 64 percent. A third period of nitrogen produced a large increase. This nitrogen period lasted 23 hours and on the introduction of air there was an increase in CO_2 production of 60.5 percent, showing that the fruits were by no means exhausted. The only time the respiratory rate of the experimental fruits went below the control, after the first nitrogen period, was when they were in air following the brief nitrogen period.

This experiment makes it very clear that when tomato fruits are deprived of nitrogen some substance is formed which later is either used in respiration or stimulates respiration in air. It is of course impossible to state which is the true state of affairs, but it will be considered that the material is actually used in the production of CO_2 and water. Even though the 23 hour period was followed by a normal rise, in air, yet it becomes very evident by referring to previous graphs that not as much CO_2 was produced as would have been in a longer period, and the 6 hour period was so short that very little material was formed. If comparisons are made between different experiments it will be evident that the more actively growing fruits produce much more of this unknown substance during intramolecular respiration than do fruits that have ceased growing and are ripening.

To check the effect of nitrogen two experiments were conducted in which hydrogen replaced air. Only one of these will be cited. It is designated as experiment 11, illustrated by text figure 11. In this experiment eight green fruits were used, having a total weight of 130.5 g. with an average diameter of 3.37 cm. and a growth increment of 6 percent per day. The control had 7 green fruits weighing 115.75 g. and growing at the rate of 6.5 percent per day. The experiment lasted 13 days from the time the fruits were put into the apparatus until the last collection was made. The first 100 hours after air was replaced were an exact duplicate of experiment 10 except that



TEXT FIG. 11. Respiration of eight green fruits, growth 6.0 percent per day. In this experiment air was replaced by hydrogen instead of nitrogen as in previous experiments. Aside from this fact, the experiment is a duplicate of experiment 10, after the introduction of hydrogen for the first 100 hours. It will be noted that when hydrogen replaces air the rise in CO₂ production is slightly higher than when nitrogen replaces air in fig. 10 (see text for explanation). In the latter part of this experiment the control had its air replaced by hydrogen for a 22 hour period.

hydrogen replaced nitrogen. Experiment 11, however, was continued for 200 hours longer.

As mentioned in another place the rise in CO_2 output when air is replaced by hydrogen is a little greater than when nitrogen replaces air, a result which is attributed to the more rapid diffusion of hydrogen. Aside from that fact there is no essential difference between experiments 10 and 11. When the hydrogen period was only six hours the rise in CO_2 was also less in this experiment than when the period was longer. A feature of this experiment is the replacement of air by hydrogen in the control near the end. When hydrogen was introduced at the 200th hour the rise in CO_2 output was not so great as in earlier replacements and an attempt was made to determine whether this was due to injury produced by the lack of air for several periods or whether it was due to natural causes operating in fruits that had been picked for some time. For this purpose hydrogen was introduced into the control respiration chamber at the 227th hour. The rise in CO_2 was even less in the control than in the experimental fruits, so the lower production was not due to any injury. Air was again admitted to the control after 22 hours in hydrogen and the rise in CO_2 elimination was a little higher than in the experimental fruits 22 hours before. This experiment also shows very conclusively that the increased CO_2 production is not due to injury, because as soon as the effect of the intramolecular respiration was over the aerobic respiration of the experimental fruits came down to the level of the control as shown at the 125-, 175-, 200-, 280-, and 305-hour periods, when it was almost identical with that of the control.

The matter of injury to the fruits when deprived of oxygen will be discussed briefly. It may be said that in general there was some injury to the fruits if the nitrogen period extended over 70 hours and when the fruits were pink the injury appeared sooner. The first appearance of injury was that of "oedema-like" watery blisters. In experiments like 10 and 11 in which the nitrogen or hydrogen periods were never over 24 hours there was no apparent injury. The fruits were examined microscopically as well as macroscopically. It was noticed that the protoplasm of the epidermal cells had contracted. The internal cells were all normal in appearance. The fruits were kept on a table in the laboratory for about two months after experiments 10 and 11, but after this length of time there was nothing in the appearance of the fruits to indicate that they had been deprived of oxygen at any time. Both groups developed an orange-red color. Fruits picked as early as these never developed a red color.

From the fact that the fruits that showed as much respiratory increase as any were not injured there can be no supposition that the CO_2 produced is a result of injury, nor does it seem possible that the increased CO_2 output could be due to increased permeability of the epidermis to oxygen resulting from the slight injury to the epidermal layer. The increases must be due to causes already discussed.

From these experiments the conclusion cannot be definitely drawn that tomato fruits do normally, *i.e.* in air, carry on intramolecular respiration. However, the hypothesis that they do is very attractive. After having been deprived of oxygen they settle down, after some hours, to a very regular and steady rate of respiration which is merely on a lower level than that in air. Otherwise, as illustrated by figures 1 and 2, fruits respiring intramolecularly are not different from the controls. In fact their respiratory rate remains more nearly constant than that of the controls. The injury mentioned is mostly to the external cells which are normally supplied with an abundance of oxygen, and not to the cells in the interior where oxygen tension must be low at all times. It seems highly probable that under normal conditions the interior respire to some extent, at least, intramolecularly, and the exterior aërobically. When oxygen is replaced the outer cells have to change their mode of respiration; the product of their respiration is poisonous to the cells and they are injured. The lowering in total respiration for a fruit may be considered as the difference between the intramolecular plus aërobic respiration in air and intramolecular alone in absence of air.

Intramolecular respiration as a normal process of various higher plants has, of course, been long known. Thus Gerber (3) stated in 1896 that there is intramolecular respiration in various fleshy fruits during the ripening processes and that this is connected with the formation of esters associated with these fruits. Devaux (2) has shown that there is a deficiency of oxygen in the interior of stems of trees and as a consequence intramolecular respiration takes place in these parts. He also believes that he has detected alcohol in such stems, under normal conditions.

The experiments here reported support these contentions to some extent, but further examinations of the gases in the interior of fruits and also an examination of the CO_2/O_2 ratio when the fruits are in air is necessary before one can definitely state that intramolecular respiration is a normal process in tomato fruits. Even if no alcohol were found intramolecular respiration might still take place, since other substances besides alcohol can be formed in intramolecular respiration.

SUMMARY AND CONCLUSIONS

1. When tomato fruits of all ages and stages of development are deprived of oxygen by substituting nitrogen or hydrogen there is usually an increase in CO_2 production.
2. This increase is followed within a few hours by a large decrease below the normal rate.
3. After this decrease the rate of CO_2 production drops only very gradually (no more than in the control) during an experiment lasting for several days.
4. Light yellow or pink fruits form an exception to this rule. Their CO_2 production drops very rapidly from the first short increase.

5. When air is again introduced there is always an increase in CO_2 production. In some fruits this increase is very large, in others small.

6. In a given fruit the amount and continuance of this increase is dependent upon the length of the period of intramolecular respiration.

7. This being so it is suggested that during the intramolecular phase of respiration substances are formed which, when air is again admitted, are rapidly oxidized to CO_2 and water.

8. The production of CO_2 in the absence of oxygen is not due to injury to the fruits, as the fruits in many experiments were in perfect condition at the end.

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A PETRIFIED LEPIDOPHYTE CONE FROM THE PENNSYLVANIAN OF MICHIGAN ¹

CHESTER A. ARNOLD

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Fossilized remains of the strobili of the Paleozoic Lycopodiales have frequently been reported but usually as flattened impressions showing little or none of the original structure. Of petrified remains structurally preserved, only two discoveries have thus far been reported in America. *Lepidostrobus Fischeri* Scott & Jeffrey (3) is a portion of a petrified strobilus from the Mississippian of Kentucky, and another specimen which has not received specific designation is reported from the Pennsylvanian of Iowa (1). The recently discovered Michigan specimen is an entire cone but neither of the others were complete.

The specimen under consideration was found by Professor H. H. Bartlett in a sandstone layer in a shale pit one mile west of Grand Ledge, Michigan, during the summer of 1929. The formation exposed at this place is considered as belonging to the Pottsville series which is low down in the Pennsylvanian system. Two coal seams, each a foot or more in thickness, are exposed in the quarry and the accompanying shales and sandstones have yielded several species of Carboniferous plants. *Stigmaria ficoides*, the root like organ of the Lepidodendreae, is abundant.

The cone, which measured 2.5×11.5 centimeters, had the general shape and dimensions of a good sized cigar (text fig. 1). It tapered rather gradually at both ends and had been flattened to about one-half of its original diameter. Judging from the base of the cone, it was originally borne on a peduncle.

Preservation of the structure is only partial. The xylem strand is intact but the phloem, the cortex, and the attachment of the sporophylls have disappeared. Also the spores had escaped and nothing remained of the sporangia except the crumpled fragments of the walls mixed with the infiltrated siliceous matrix. Since most of the structure of the sporophylls had disappeared and the sporangia had collapsed, very little could be determined concerning the morphology of these parts. However, decay did not take place until the enclosing matrix had partly solidified which resulted in the formation of cavities in the rock mass which were formerly occupied by the bracts. The cavities corresponding to the free upturned laminae on the distal portion of the stipes are well shown on the upper portion of the cone. On the lower portion the outside is broken away so as to show the

¹ Paper from the Department of Botany, University of Michigan, No. 343.

ends of the stipes. The enclosing matrix consists of sand grains cemented together with precipitated silica.

Judging from the areas formerly occupied by sporangia, it appears that the latter were approximately five millimeters long and probably less than half as high. Their width is unknown. The stipitiform portion of the

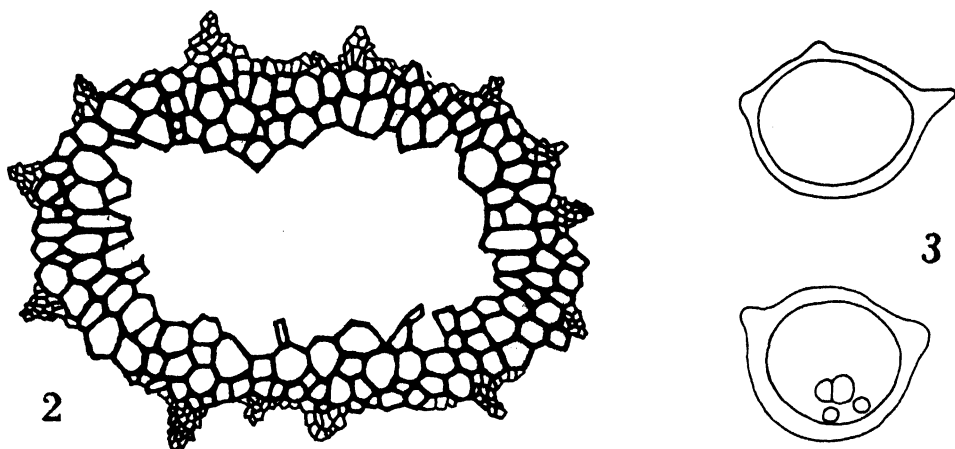


TEXT FIG. 1. Photograph of cone, natural size. The central part was ground away to expose the axis.

sporophyll which seems to have been borne at right angles to the axis appears to be a centimeter or less in length, and the free portion of the lamina appears to have been about the same or possibly slightly longer. The sporophylls are spirally arranged as shown by the dots on the surface of the axis where the sporophyll traces emerged.

The sporangium is attached by a narrow base to the broad upper surface of the sporophyll. Extending upward into the sporangial cavity from the base is the radial plate of sterile tissue which is frequently observed in sporangia of this type. The walls of the sporangia are made up of a single layer of prismatic palisade cells which are characteristic of *Lepidostrobus* sporangia.

Heterospory is indicated in this specimen by remains representing both the megaspores and the microspores. Only two of the former were observed, one of which contains four oöspore-like bodies of a fungus (text



TEXT FIG. 2. Cross section of the xylem cylinder. $\times 180$. TEXT FIG. 3. Megaspores. The lower one contains fungous bodies. $\times 40$.

fig. 3). In shape, the megaspores are broadly oval and measure about 735 microns along the largest diameter. Two or three low ridges are visible on the surface of the thick wall but they do not bear long appendages as do some lepidodendroid spores. The smaller structures which appear to be microspores are about 20 microns in diameter. They are not well preserved and their identification as microspores is not positive but the presence in the same cone of spores too large to be the spores of a homosporous plant render its heterosporous nature quite certain. Since none of the spores are still enclosed in sporangia it is impossible to determine in what portion of the strobilus the different kinds of spores were borne.

The xylem strand of the cone axis is about one-half of a millimeter in diameter (text fig. 2). The center consists of a pith which is surrounded by scalariform tracheids. The inner zone of rather large tracheids is two or three cells in extent and outside of this is a narrow discontinuous zone of smaller tracheids which is rather sharply set off from the inner zone. Projecting outward from this zone are numerous protoxylem points. It is impossible to determine whether the sporophyll traces depart from these points or from the spaces between.

Concerning the affinities of this fossil, the poor preservation renders comparison difficult. The cones commonly referred to *Lepidostrobus* (the isolated and unattached cones which resemble those of *Lepidodendron*) are characterized by the attachment of the sporangium all along its lower side to the upper surface of the stipe. This feature could not be observed in our specimen. However, there are other features displayed which have much in common with *Lepidostrobus* and which may be tabulated as follows: *a*, the typically lepidodendroid xylem strand; *b*, the apparent heterospory; *c*, the cells of the sporangial wall; *d*, the attachment of the sporangium by a narrow base; *e*, size and external appearance.

Since it is not well to lay stress on the absence of similarities when comparing imperfectly preserved specimens one is often confronted with the problem of whether to multiply species at the risk of duplication, or to refer specimens to species already described but with which they do not closely agree, or to leave them without specific designation. The disadvantage of the latter course is obvious. A specimen not receiving specific recognition and consequently lacking a convenient name is soon lost to science. Williamson (4), when describing *Lepidostrobus Oldhamius*, pertinently remarked:

I have for many years endeavored to discover some specific character by which different *Lepidostrobili* could be distinguished and identified, but thus far my efforts have been unsuccessful. . . . I continue to shrink from giving specific names to examples which, in all probability, only represent forms reappearing in more than one species; but, as in a previous memoir, I still recognize the opposite inconvenience of having no concise means of referring to any object figured. With a distinct understanding as to the meaning and purpose of such names, and since the *Lepidostrobus* under consideration is characteristic of the Oldham deposits, I will designate the type *L. Oldhamius*.

While showing the same general structure as *L. Fischeri* and the unnamed species from Iowa, certain differences are at once apparent, the outstanding one being size. The Iowa specimen, which was complete except for the tip, was 22 centimeters long, or over twice the length of our specimen. It is described as having but one kind of spore, but whether this species was homosporous or merely dioecious is unknown. *L. Fischeri* is described by the author as closely resembling *L. Brownii*, which is quite different from our fossil. The diameter of the axis in our specimen seems to agree fairly well with that of *L. Oldhamius* but there are certain differences in the xylem which seem to separate the two forms taxonomically. Also, nothing is known concerning the length of *L. Oldhamius*. Our species seems to agree also in a general way with the somewhat smaller cone, *L. Veltheimianus*, but this form has a very small pith and megaspores which are different. It might be well to call attention to the impression of the strobilus of *Lepidodendron Sternbergii* Brong. (2), as figured by Lesquereux. While the figure gives no adequate idea of the surface features of the cone, it shows a remarkable similarity in size and shape to our specimen.

It is apparent that there is a general agreement between our fossil and

several of the previously described forms and that it shows no pronounced differences. At the same time it shows details which render its identity with the above mentioned species doubtful. So because of our very meager knowledge on American *Lepidostrobus*, and in order that this specimen be placed on record, it is hereby named ***Lepidostrobus Bartletti*** sp. nov., after its collector.

The type sections have been catalogued as no. 12862 in the Museum of Paleontology of the University of Michigan.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MICHIGAN

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CHROMOSOME NUMBERS IN CULTIVATED CUCURBITS

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The primary purpose of this investigation was to determine the chromosome number of the various species in the group of cultivated cucurbits with the idea in mind that a cytological study of the different members would serve as a valuable adjunct in a genetical analysis to be made later.

The material does not lend itself easily to this type of study owing to the meagre amount of chromatin material and the correspondingly small and numerous chromosomes.

MATERIAL AND METHODS

The material used in connection with this investigation consisted of seed secured from commercial seed houses and inbred one generation.

The observations were made upon commercial varieties of *Cucumis sativus* (cucumbers), *Cucumis Anguria* (gherkins), *Cucumis Melo* (melons), *Citrullus vulgaris* (watermelons), *Lagenaria vulgaris* (gourds), *Cucurbita Pepo* (summer squash), *C. moschata* (Cushaw and winter crook-neck squash), *C. maxima* (Hubbard squash).

Several fixing solutions were tried. Bouin's fluid and Allen's modification of Bouin's fluid proved to be the most satisfactory ones. The latter was especially good in the fixation of root tips where rapid penetration is essential. Weak chromo-acetic solution (Schaffner's formula) and Flemming's weak chromo-acetic-osmic were tried but they were not as satisfactory as the former two. The material was dehydrated, cleared in xylol, and imbedded in paraffin.

The sections were cut from $5\ \mu$ to $7\ \mu$ in thickness and were stained in Heidenhain's iron-alum haematoxylin.

After the young flowers had been gathered the anthers were dissected out and tested by making a smear preparation of a small portion of the material. This smear preparation was stained with methyl green (1 percent solution in water, acidulated with acetic acid). By examining a smear preparation under the microscope and using this technique, it is possible to tell whether the microspore mother cells are in the process of division. In case the microspore mother cells were found to be dividing, the remaining portion of the anther was fixed in Bouin's fluid or Allen's modification of Bouin's fluid.

The most desirable time to fix the young buds is from 3 A.M. to the time of anthesis of the older flowers. Anthesis ordinarily takes place from 5 A.M. to 7 A.M. in the locality in which the work was done. In *Lagenaria vulgaris* where anthesis takes place from 6 P.M. to 7 P.M. the most desirable

time to fix the young buds is from 4 P.M. up to the time of anthesis. An examination of the material of all types indicates that at all other times of the day the microspore mother cells are apparently in a resting condition.

The root tips were fixed with Allen's modification of Bouin's fluid. Using this fixative and staining with iron haematoxylin for 24 hours, the chromosomes were very distinct with the minimum amount of clumping. On the contrary when Bouin's fluid was used the chromosomes were more or less lumped and indistinct. The same was true when fixation with weak chromo-acetic (Schaffner's formula) was tried.

In order to determine the time best suited to fix the root tips so as to secure the greatest amount of cell division, material was fixed during all hours of the day. The period from 4 A.M. to 5 A.M. gave the best results. During this period an abundance of cells were found undergoing division. Opposed to this, the period from 10 A.M. to 12 A.M. exhibits a minimum amount of cell division.

The material used during the course of this investigation was grown under field conditions at the Blandy Experimental Farm.

LITERATURE

The amount of cytological work that has been done with members of this group is very small, which is astonishing when one considers their economic importance and world wide distribution.

In reviewing the literature, there appears to be some confusion in regard to the chromosome number of the various species in the cultivated genera of the Cucurbitaceae.

The most extensive study that has been made of this group was reported by Kozhukhow (1925). He gives the following list as a result of his study:

| Species | Diploid Chromosome No. |
|--|------------------------------|
| <i>Cucumis sativus</i> L. | 14 |
| <i>Cucumis Melo</i> L. var. gr. <i>melitensis</i> Alef. | 24 |
| <i>Citrullus vulgaris</i> L. | 22 |
| <i>Cucurbita Pepo</i> L. var. gr. <i>pomiformis</i> Alef. | 40 |
| <i>Cucurbita Pepo</i> L. var. gr. <i>citrullina</i> Alef. | 42 |
| <i>Cucurbita maxima</i> Duch. | 48 |
| <i>Cucurbita moschata</i> Duch. | 48 |

These observations were made from root tip material and were not checked by similar observations from the microspore mother cells.

Castetter (1926) described microsporogenesis in an inbred strain of the Hubbard squash (*Cucurbita maxima*). He found that this strain had twenty haploid chromosomes and forty diploid ones. The diploid count was made from root tip material.

Perhaps the most interesting feature of this study was the appearance of cell plates in both the heterotypic and homotypic spindles. They do not appear to take any part in the division of the pollen mother cells. The

presence of cell plates has been noted by earlier workers and they were assumed to take part in the quadripartition of the pollen mother cells, but according to Castetter in *C. maxima* they are reabsorbed without apparently serving any function.

Heimlich (1928) investigated microsporogenesis in *Cucumis sativus*. He reports the haploid chromosome number as seven and the diploid as fourteen. His observations were made upon microspore mother cells, anther primordia, and root tip cells. Unlike Castetter he did not observe cell plates during meiosis. It is very probable that the details of meiosis in *Cucumis sativus* differ slightly from those which take place in *Cucurbita maxima*.

In the discussion of his observations, Heimlich (1928) states that budding nucleoli were frequently observed. These budding nucleoli behave very peculiarly just before disappearing, becoming vermiform with usually one globular end. If they are associated with the chromosome group, they may be mistaken for chromosomes. This latter point may account for some of the confusion in the literature in regard to the number of chromosomes reported by various workers for members of this group.

Lundegårdh (1914, according to Jones and Rosa, 1928) gives the chromosome number in *Cucurbita Pepo* as twelve haploid, twenty-four diploid. It was not stated from what source the material was secured. These numbers differ widely from those reported for *C. Pepo* by Kozhukhow (1925) and Castetter (1930). The cause of these widely diverging numbers is problematical and the present writer cannot suggest an explanation.

Castetter (1930) counted the chromosomes in *C. Pepo* and *C. moschata* in connection with a study made upon species crossing in *Cucurbita*. In *C. Pepo* var. Connecticut Field, he found the haploid number to be twenty and the diploid forty. In *C. moschata* the haploid number was twenty-four and the diploid forty-eight. He states that "no difference in size or shape of chromosomes was noticeable in these three species, nor was it possible to find any other cytological distinction between these species with respect to microsporogenesis and nuclear division of the root tip."

The following table briefly summarizes the chromosome work that has been done with the cultivated cucurbits:

| Species | Investigator | Haploid Chromosome No. |
|--|--------------|------------------------------|
| <i>Cucumis sativus</i> | Kozhukhow | 7 |
| <i>Cucumis sativus</i> | Heimlich | 7 |
| <i>Cucumis Melo</i> | Kozhukhow | 12 |
| <i>Citrullus vulgaris</i> | Kozhukhow | 11 |
| <i>Cucurbita Pepo</i> var. <i>pomoformis</i> | Kozhukhow | 20 |
| <i>Cucurbita Pepo</i> var. <i>citrullina</i> | Kozhukhow | 21 |
| <i>Cucurbita Pepo</i> | Castetter | 20 |
| <i>Cucurbita Pepo</i> | Lundegårdh | 12 |
| <i>Cucurbita maxima</i> | Castetter | 20 |
| <i>Cucurbita maxima</i> | Kozhukhow | 20 |
| <i>Cucurbita moschata</i> | Kozhukhow | 24 |
| <i>Cucurbita moschata</i> | Castetter | 24 |

OBSERVATIONS

In this paper no attempt will be made to describe and discuss the details of microsporogenesis in the various species. The writer simply seeks to record the number of chromosomes found in the material during the course of the investigation and mention some of the most obvious changes taking place during meiosis.

Cucumis sativus

The haploid number of chromosomes in this species is seven. (Pl. LXIII, fig. 1.) This is indicated by numerous counts of the chromosomes contained in the pollen mother cells at both the heterotypic and homotypic divisions. Cells from the rapidly growing root tip primordia contain fourteen chromosomes.

The most favorable place to make the counts is during the heterotypic division at the equatorial plate or the metaphase stage. A polar view of the metaphase stage during this division will show the chromosomes well spread out, distinct and clear in most cases. These observations in regard to the chromosome number of *C. sativus* are in agreement with those of other workers (Heimlich, 1928; Kozhukhow, 1925).

There appears to be no evidence of cell plate formation between the heterotypic and homotypic divisions. During the heterotypic division, the two spindles are invariably at right angles to each other.

Examination of the material reveals the fact that all of the pollen mother cells of an individual flower bud are in practically the same stage of development. The microspore mother cells of an individual usually exhibit some phase of the reduction division or of the equational division. Rarely have flowers been examined in which both divisions were represented. There is not, on the other hand, any variation in the stage of development of the pollen mother cells from the lower to the upper portion of the anthers.

Division in the pollen mother cells apparently does not take place any later than 9 A.M. under field conditions. Thus it seems probable from a consideration of the above mentioned facts that meiosis in pollen mother cells takes place very rapidly. The reduction division is evidently followed immediately by the equational division, the whole process probably requiring several hours to go to completion.

These are generalizations which can be made not only in regard to *C. sativus* but to all of the species that have been studied with the exception of *Lagenaria vulgaris*.

Short Green Gherkin was the variety of *C. sativus* used in making the study. Material from several other varieties, namely Henderson's and Everbearing, was examined and the haploid number found to be seven.

Cucumis Anguria

Numerous counts of the chromosomes of the pollen mother cells in this species show that the haploid number is eleven (fig. 2). In the cells of the root tip, mitotic figures were found where the chromosomes at the equatorial plate stage were sufficiently spread out to make counting possible. The diploid number is undoubtedly twenty-two.

In this species, owing to the comparatively small size of the flower buds, it is extremely difficult to make preparations showing division figures with the ordinary technique described previously. The buds are not large enough to furnish material for both a smear and a fixed preparation but by using a trial and error method it was possible to secure very good mounts showing the pollen mother cells during both the reduction and equational divisions.

There are not any well marked morphological differences as far as the size and shape of the chromosomes are concerned.

The variety of *C. Anguria* used for study was West India Gherkin.

Cucumis Melo

Chromosome counts made from pollen mother cells of this species show twelve haploid chromosomes (fig. 3). The diploid number counted in the root tip cells is twenty-four.

In *C. Melo*, meiosis takes place in the usual manner, no irregularities having been observed. There apparently are not any distinct individual differences in the chromosomes.

The variety Lake Champlain was used as the source of material in making chromosome counts of *C. Melo*.

It will be noticed that in this genus (*Cucumis*) of the Cucurbitaceae the chromosome numbers of the three recorded species do not form a polyploid series, as is common among many other genera of plant families, notably *Solanum* and *Nicotiana*. The numbers 7, 11, and 12 apparently have no relation to one another. They certainly cannot be accounted for on the basis of a doubling of the chromosome number or by non-disjunction of one of the bivalents. However, there are great morphological differences between members of the three species and cases of species hybrids have never been reported in the literature. The present writer has repeatedly tried interspecific crossing without success, so it may be assumed that so far as we know they are cross sterile.

In members of this genus evidences of "ephemeral" cell plates described by Castetter (1926) for *Cucurbita maxima* were not observed. These cell plates do not appear after either the heterotypic or homotypic divisions. The quadripartition of the tetrad nuclei takes place by a furrowing or invagination of the plasma membrane to form the new cell wall.

Citrullus vulgaris

The haploid number of chromosomes of *C. vulgaris* variety Tom Watson is eleven (fig. 4). In the Radio variety the haploid number is also eleven. The diploid number as evidenced by counts of the root tip cells is 22. Kozhukhow (1925) also found twenty-two chromosomes in the root tip cells.

The meiotic divisions take place in the ordinary manner. During the homotypic division the spindles are sometimes formed at right angles to each other, while in other cases they are parallel. This is somewhat different from the members of *Cucumis* where the spindles are always at right angles to each other.

Lagenaria vulgaris

The haploid and diploid number of this species was found to be eleven and twenty-two, respectively (fig. 5). The chromosomes of this species are fairly large ($1.5\ \mu$) compared with those of *Cucumis* and *Citrullus* ($1\ \mu$). The pollen mother cells are also relatively larger so it is comparatively simple to find cells where counting can be done very easily.

In this species there is evidence of cell plate formation immediately after the late telophase of the heterotypic division. A complete wall is not formed and the cell plates disappear as soon as the prophase stages of the homotypic division are initiated. Cell plates have also been observed after the tetrad nuclei have been formed, but they do not play a part in the formation of the wall which surrounds the mature pollen grains.

These observations were made on the variety African Pipe gourd.

Cucurbita Pepo

In this species the particular variety used (Winter Luxury) has twenty as the haploid number. The root tip material is very difficult to work with because of the relatively small amount of chromatin material and the large number of chromosomes. However, by making a number of counts the diploid number can be definitely placed at forty.

The meiotic divisions take place in the customary fashion. The chromosomes are approximately of the same size and shape.

"Ephemeral" cell plates described by Castetter (1926) appear during the late telophase stage of the heterotypic division just before the spindle fibers disappear. These cell plates are very distinct, forming practically a continuous wall between the two nuclei of the pollen mother cell. They do not, however, extend into the cytoplasm.

During the earliest stages of the homotypic division these cell plates disappear and are not visible again until after the completion of the homotypic division where they show up very distinctly across the equator of the spindle. They disappear again just previous to the quadripartition of the pollen mother cell.

Cucurbita maxima and *Cucurbita moschata*

The diploid number of chromosomes in *C. maxima* counted in the root tip cell is forty and for *C. moschata* forty-eight. However, these observations have not been verified by a similar set of observations upon microspore mother cells. The varietal forms of *C. maxima* and *C. moschata* used were Mammoth Chili and Calhoun, respectively.

SUMMARY

The following table gives the chromosome number of the various species under observation during this study:

| Species | Chromosome Pairs |
|---------------------------------|------------------|
| <i>Cucumis sativus</i> | 7 |
| <i>Cucumis Anguria</i> | 11 |
| <i>Cucumis Melo</i> | 12 |
| <i>Citrullus vulgaris</i> | 11 |
| <i>Lagenaria vulgaris</i> | 11 |
| <i>Cucurbita Pepo</i> | 20 |
| <i>Cucurbita maxima</i> | 20 |
| <i>Cucurbita moschata</i> | 24 |

The work was done under the direction of Dr. O. E. White to whom the writer wishes to express his thanks for advice and criticism.

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DESCRIPTION OF PLATE LXIII

The drawings were made with a Zeiss microscope equipped with a 2 mm. apochromatic objective and 20 × and 10 × oculars. An Abbe camera lucida was used in all instances. The magnification of figures 1 and 4 is 1800. In the remainder of the figures the magnification is about 3200.

FIG. 1. *C. sativus* showing polar view of the seven haploid chromosomes at the heterotypic division.

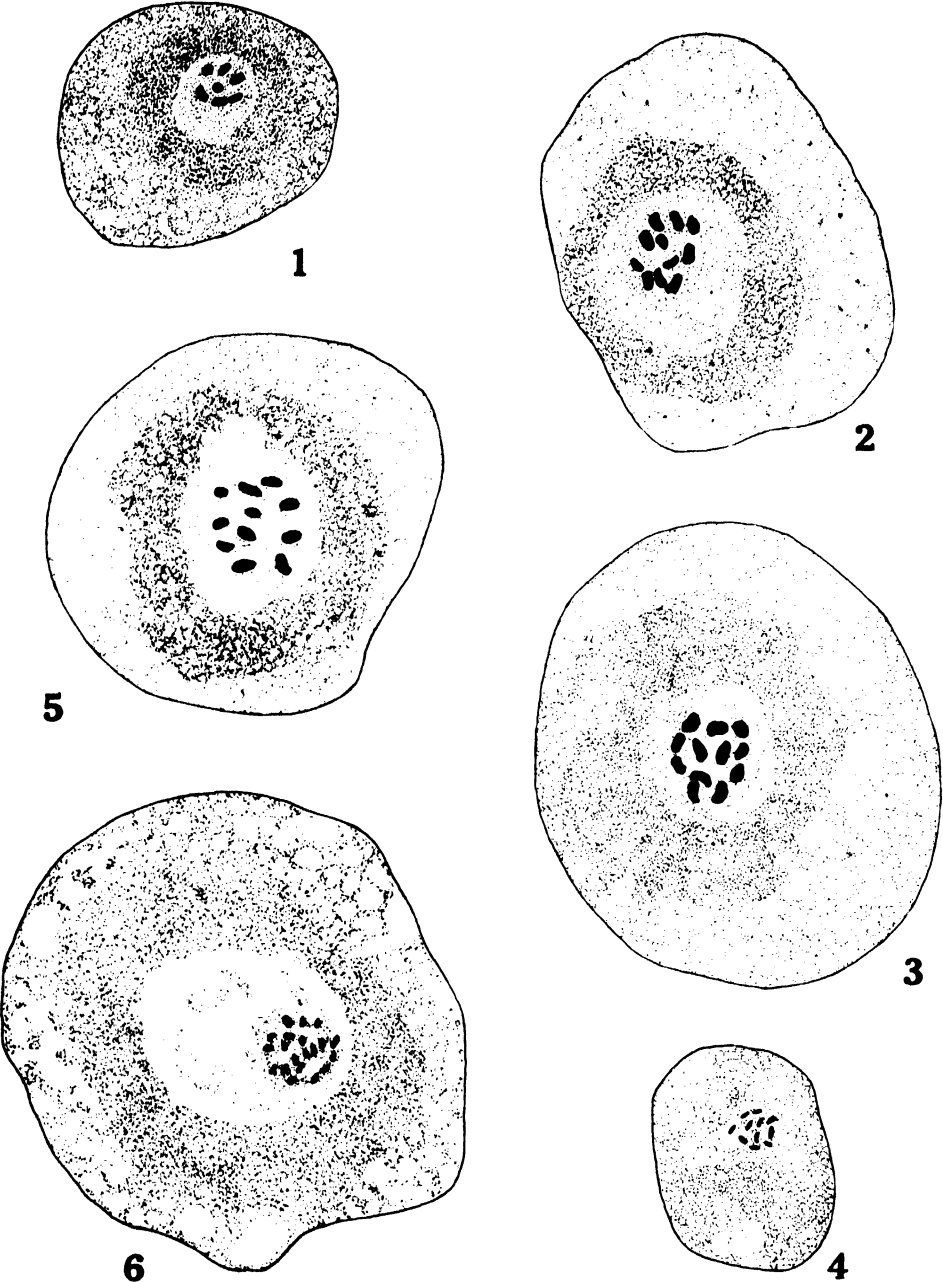
FIG. 2. *C. Anguria* showing the eleven haploid chromosomes. A polar view of the metaphase stage of the heterotypic division.

FIG. 3. *C. Melo* showing twelve chromosomes in the heterotypic division. A polar view of the metaphase stage.

FIG. 4. *Citrullus vulgaris* showing eleven chromosomes in the heterotypic division, a polar view at the metaphase stage.

FIG. 5. *Lagenaria vulgaris* showing twelve chromosomes at the heterotypic division. An equatorial plate stage.

FIG. 6. *Cucurbita Pepo* showing twenty haploid chromosomes. A polar view of the equatorial plate stage of the heterotypic division.



WHITAKER: CUCURBIT CHROMOSOMES

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ABSTRACTS OF THE PAPERS PRESENTED BEFORE THE PHYSIOLOGICAL SECTION OF THE BOTANICAL SOCIETY OF AMERICA, CLEVELAND, OHIO, DECEMBER 30, 31, 1930; AND JANUARY 1, 1931 *

The Responses of Longleaf Pine (*Pinus palustris* Miller) Defoliated by Fire and Turpented for Three Years. *Eloise Gerry, Forest Products Laboratory (maintained at Madison, Wisconsin, in coöperation with the University of Wisconsin), Forest Service, U. S. Department of Agriculture.*—In a young South Carolina longleaf pine stand a light winter fire occurred in 1927. Little was burned except the surface litter, but in a small area the fire became very hot, scorching and killing the entire foliage, except the leaf buds, on a number of trees. A group of 10 scorched trees (9 inches d.b.h.) were matched with similar unscorched trees close by, since detailed information on the effects of fire on oleoresin production was lacking. In 1927 less than half the normal yield of oleoresin was obtained from the burned trees. Throughout the year they showed, in the tissues above the face, not only a delayed and incomplete development of wood cells, but also a reduced amount of both wood (especially summerwood) and resiniferous tissues, as compared with the unscorched trees. By mid-season three trees were dead and one dry-faced. Another, though living, became unproductive by the end of 1927. Finally less than 50 percent of the productive power of the original stand remained. In 1928 and 1929, however, the surviving trees showed a remarkable recuperation as indicated by their new crowns of leaves, their high yield of oleoresin, practically equaling tree for tree that from the check trees, and the improved wood formation which, nevertheless, was still markedly less than that of the check trees. (This work was done in coöperation with the Southern Railway, owners of the timber, and Dr. Austin Cary of the Forest Service, Washington, D. C., who selected the trees.)

A Comparison of the Stimulating Effect of Commercial Fertilizers on Sugar Utilization by a Plant. *D. J. Verda, G. C. Wickwire, and W. E. Burge, University of Illinois, Urbana, Ill.*—Fifty-seven different kinds of commercial fertilizers were used in this investigation. Large quantities of *Spirogyra porticalis* were collected and after squeezing gently with the

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hands to remove the excess water, batches of 150 grams were weighed out. These were placed in 600 cc. of 0.1 percent dextrose solution in flat bottomed dishes 20 cc. in diameter. Six-tenths grams of the fertilizer, after being heated in 10 cc. of water, were added to the solutions. Sugar determinations were made of the solutions according to the method of Benedict immediately and after 48 hours at the end of the experiments. It was found that all the fertilizers increased the rate of sugar utilization but that the nitrogen-rich fertilizers increased it most. It is known that the nitrogen-rich foods, the proteins, stimulate metabolism most strongly in animals and in this respect it would seem that animals and plants are alike. It is known that the phosphates also exercise a strong stimulating action on sugar metabolism in animals but in this investigation it was found that this is not true of plants, and in this respect animals and plants differ.

Greenhouse Plants and Illuminating Gas. *P. W. Zimmerman, Wm. Crocker, and A. E. Hitchcock, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—A large number of plants which were subjected to various concentrations of illuminating gas varied in sensitivity, type of response, and the particular organs affected. Characteristic responses were the epinasty of leaves, yellowing of oldest leaves, and abscission of leaves and flowers or flower buds. Previous workers have shown that the same type of response is produced by ethylene. Maximum curvature for tomato leaves occurred at or above 75° F. and decreased with decreasing temperature until at about 50° F. no typical response was noted. Inverted tomato plants exposed to illuminating gas failed to give an epinasty typical for upright plants, the degree of curvature being much less. Roses subjected to 200 p.p.m. for 7 days showed no injury at 45–50° F. Similarly treated plants at 75° F. showed yellowing and abscission of leaves after an exposure of only 48 hours. These plants as well as those exposed to 100 p.p.m. eventually lost most of their leaves. Later a complete recovery was characterized by the active growth of all latent buds. In the case of untreated plants that were hand-defoliated a normal response resulted in which only a few of the buds produced an active growth.

The Effect of Sulphur Dioxid Fumes on Plants. *P. W. Zimmerman and Wm. Crocker, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—Sulphur dioxid injured the foliage of all the plants tested. Injury in the form of brown or withered areas usually appeared first along the margins of the leaves, then between the veins. Regions along the main veins frequently resisted high concentrations. After a one hour treatment in 3–4 p.p.m. of SO₂ leaves were injured on tomato, salvia, coleus, geranium, castor bean, roses, and several other plants. The same plants in a slightly wilted condition were not injured by 8 p.p.m. after a five hour treatment. Middle-aged leaves were more sensitive than either young or old leaves. Orchid foliage showed no injury after a four hour treatment in 70 p.p.m.,

but a high concentration for a longer period injured the leaves and some of the exposed roots.

The Effects of Essential Metals on Injection into Plant Protoplasm and Sap. *Thomas Kerr, University of Pennsylvania, Philadelphia, Pa.*—The effects of the chlorids of K, Ca, and Mg on the protoplasm and the vacuole of the root hairs of *Trianea bogotensis* were studied by injecting these salts with the use of the Chambers micromanipulator. The active protoplasmic streaming served as a criterion of normality. KCl increases the fluidity of the protoplasm when injected into it, and also produces a harmful effect on the ability of the protoplasm to repair its surface at the site of injection. When injected, CaCl_2 produces a solidification of the protoplasm which tends to become localized. The injected region persists as a local coagulum, and streaming is resumed in the rest of the cell. CaCl_2 and KCl are mutually antagonistic in the interior of the protoplasm. MgCl_2 produces a gradual solidification of the entire protoplasm when injected. In the vacuole, KCl and MgCl_2 injections give little perceptible effect. On the other hand, CaCl_2 reacts with the vacuolar contents to form a precipitate, presumably of calcium oxalate crystals. These crystals can be seen even upon the injection of .003 M. Sodium oxalate produces no harmful effect when injected into the vacuole although extremely toxic upon immersion.

Seasonal Variations in the Hydrostatic-Pneumatic System of Certain Trees. *J. B. Overton, University of Wisconsin, Madison, Wis.*—MacDougal, Overton, and Smith have shown that the movement of sap in *Alnus* is through the spring wood, that in *Salix* through the late summer wood, and that in *Juglans* through the early spring and late summer wood. The portions of each annual layer of wood which were not concerned in the transport of sap were found to be filled with gases. There seems to be a marked seasonal variation in the width of the water-containing portion of each conducting layer, but the results were not particularly conclusive because their work dealt mainly with mid-summer conditions in California. Since the close of the above-mentioned work I have been able to follow throughout the season in Wisconsin the variations between the water conducting and gas-filled portions of *Alnus incana* and of several species of willow. During the summer and autumn, while the leaves were still on the trees, the distribution of the transpiration stream and its relation to the gas-filled portions of each annual ring were exactly as described for the California species. As the season advances and transpiration is reduced toward winter the gas-filled portion of each annual layer becomes narrower and by spring is almost or completely filled with water. However, in the willow and alder some vessels or groups of vessels seem never to be entirely emptied of gas and filled with water but retain their gas content throughout

the season. As spring advances the zone of conduction decreases in width, finally assuming the picture presented in mid-summer.

Studies in the Growth of Cotton Fibers. *Wanda K. Farr, Division of Cotton Marketing, U. S. Department of Agriculture, Washington, D. C.*—Fiber formation is first confined to the epidermal cells of the seed coat. The elongation of these fibers and the increase in size of the other tissues of the seed are the forms in which growth is expressed until the twelfth day, when fiber formation from the subepidermal layers begins. These are easily distinguished from the first growth of fibers and normally constitute the bulk of the entire fiber mass. Subepidermal cells continue to initiate fiber development until the period of wall thickening begins, when the process of cell elongation appears to be greatly decreased if not terminated. Fibers of epidermal origin thicken their walls very slightly or not at all. Subepidermal tissue development results in their partial or complete detachment from the other tissues of the seed, thus altering the nutritional conditions. Fibers of subepidermal origin vary in length according to the time of initiation of elongation, but thicken their walls simultaneously. The cross sectional area of the fiber base and the relative surface area of the inner wall are important factors in determining the final wall thickness, the shorter fibers of large diameter consistently developing thicker walls than long fibers of the same diameter and fibers of similar length and smaller diameter. This may be a mass expression of dehydration of glucose to form the cellulose of the wall. Measurements of large numbers of fibers from each daily stage of development are used to delimit and photomicrographs are used to illustrate the various periods of growth.

The Effect of Storage on the Vitality of Delphinium Seeds. *Lela V. Barton, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—Experiments with two-year-old, one-year-old, and fresh seeds of annual and perennial delphinium were begun in December, 1926. Original tests of these seeds indicated that a constant temperature of 15° C. and a daily alternating temperature of 10° C. to 20° C. were most favorable for seedling production. Germination tests after periods of storage up to 39 months also demonstrated the effectiveness of these temperatures. Samples were stored in open and sealed glass vials at room temperature, at approximately 8° C., and at approximately - 15° C. None of these temperatures was constant. Germination tests to determine the viability of these stored seeds were made at intervals of two to seven months. Results obtained indicated that: (1) At any temperature and especially at room temperature sealed storage was much more effective than open storage. This advantage became more marked after the seeds were two years old; (2) The cold temperatures were superior to room temperature for retaining vitality of the seeds especially in the case of open storage; (3) Seeds of perennial

delphinium deteriorated more rapidly in storage than seeds of the annual plant.

The Root Systems of Trees in Sphagnum Bogs. *Geo. B. Rigg and E. S. Harrar, University of Washington, Seattle, Wash.*—This paper reports the character and distribution of the roots of coniferous trees growing in bogs whose surface layer is composed of raw *Sphagnum*. The investigation was largely promoted by the fact that wind-falls are extremely rare in the bogs of the Pacific Northwest, although they are common on both muck and glacial tills in the same region. Six species in all, namely, *Picea sitchensis*, *Pinus contorta*, *Pinus monticola*, *Pseudotsuga taxifolia*, *Thuja plicata*, and *Tsuga heterophylla* were subjects of the study. Control studies were undertaken with certain of the species, thus rendering a basis for comparison. The roots of all species examined were confined to the upper layer (raw *Sphagnum*). Tap roots were generally wanting in species growing in *Sphagnum*. Short, stubby, tap roots were occasionally observed. Their vertical penetration was controlled by the level of the water-table. The lateral roots of trees growing in *Sphagnum* are much longer than those of equally large trees growing in glacial till. Root branching was generally less noticeable in the trees growing in the bogs. The fusion of main laterals and branches of the same root system occurred with seeming regularity in all species of the bog habitat. This phenomenon was not manifested in glacial till. The general transverse sectional slope of roots grown in *Sphagnum* is characteristic. Instead of the more usual oval configuration, these roots present rectangular, I-beam or T-girder structures. The increased mechanical efficiency of these organs is accordingly obvious.

Seasonal Distribution of Reducase in the Various Organs of an Apple Tree. *S. H. Eckerson, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—A series of weekly determinations of the nitrate-reducing activity in the different organs of a single tree was begun March 31, 1930. Through the coöperation of the New Jersey Experiment Station, especially of Dr. G. T. Nightingale, the material for the study has been obtained each week from a fruitful Stark apple tree. During the year there have been four rather definite periods in the distribution and the intensity of reducase activity. (1) When the buds were beginning to swell, both buds and fine roots were high in reducase, while the bark and the larger roots contained a little. (2) When the blossom buds were opening reducase was high in pistils and stamens, peduncles, and new stems. It was lower in the leaves. (3) From May 26 to June 16 no reducase could be found in any of the organs. Additional material from another Stark tree was tested June 9 but there was no nitrate-reducing activity. (4) In July and August the fine roots, leaves, and fruits contained a little reducase. During September it increased in the fine roots while it decreased in the leaves. In October reducase was highest in the fine roots, though much lower than

in the spring, and there was again some reducing activity in the bark and the terminal buds.

The Effects of Salts on the Extensibility of Protoplasm. *William Seifriz and Janet Plowe, University of Pennsylvania, Philadelphia, Pa.*—The chlorids of Na, K, and Li lower, while the chlorids of Ca and Ba raise the elastic limit (extensibility) of protoplasm. Mg causes no change over that of the control, sucrose. The following series results:

Ca > Sr > Mg > K > Li > Na.

The elastic limits were determined by stretching protoplasmic strands from the plasmolyzed protoplasts of *Allium* epidermal cells, with the aid of mechanically controlled micro-needles. Al greatly increases protoplasmic extensibility; part of this increase is due to change in acidity and part to the Al ion itself. HNO₃ of the same pH value (3.7) as the Al solution, causes half the increase in elastic limit as does AlNO₃. Extensibility and related phenomena such as the form of plasmolyzed protoplasts and the presence of strands from protoplast to cell wall, are not reliable criteria of protoplasmic consistency. Structure (internal cohesion) and not viscosity or surface tension, determines the elastic limit.

A Microchemical Study of Soybeans during Germination. *Floyd W. Von Ohlen, Ohio State University, Columbus, Ohio.*—Manchu soybeans were germinated in moist quartz sand and microchemical tests were made for carbohydrates, proteins, oil, and minerals on various parts of the seedlings at different stages of germination. The cotyledons of the mature soybean seeds contained a large amount of protein and oil, some non-reducing sugar, a small amount of starch, considerable organically bound phosphorus and magnesium, and some potassium. During germination the first changes detected were the appearance of reducing sugar, and an increase of starch in the hypocotyl, starch in the root cap, and an increase of starch in the cotyledons. During the first three days of germination a large amount of starch accumulated in the apex of the hypocotyl, while reducing sugar accumulated at the base of the hypocotyl and root. The amount of starch in the cotyledons increased until the fifth day, and then remained constant until the ninth day, after which there was a rapid decrease. It disappeared from the palisade tissue last. The depletion of the oil in the cotyledons began at the base and progressed toward the opposite end. The palisade tissue was depleted of oil much more slowly than were the other tissues. As the seedlings developed, there was a gradual change of the organically bound phosphorus and magnesium of the cotyledons to the inorganically bound form. At no time were starch, reducing sugar, asparagine, oil, and inorganically bound phosphorus detected in the meristematic tissue of the root tip.

The Carbohydrates of Healthy and Mosaic Tobacco Leaves. *A. A. Dunlap, Connecticut Agric. Exp. Sta., New Haven, Connecticut.*—Quantitative analyses were made of certain carbohydrate materials in healthy and mosaic tobacco plants, samples of which were taken at different times of the day and after prolonged periods of darkness and of low temperature. The amounts of reducing sugars, disaccharids, total sugars, dextrin, starch, and hemicelluloses were determined in the analyses. A reduction in all these forms of carbohydrates was found accompanying the diseased condition. This reduction was found in all mosaic plants regardless of the time of day when the samples were taken and regardless of the environmental conditions to which the plants were exposed. The amounts of reducing sugars, disaccharids, and starch apparently showed greater variations than the other forms of carbohydrates in the diseased plants. Studies were also made of the amounts of carbohydrates in the light and dark areas of the mosaic leaves.

Manganese, an Essential Element for a Green Alga. *E. F. Hopkins, Cornell University, Ithaca, N. Y.*—In connection with my studies on the relation of iron to *Chlorella* sp. I have found that manganese also is essential for growth. Increases of from 10 to 600 fold in growth have been obtained by the addition of one part of manganese in five million parts of culture solution from which the manganese had been removed. Experiments were carried out at two acidity values: pH 7.0 and pH 8.0. The results were more striking at pH 8.0, probably due to the greater difficulty of removing manganese impurities from culture solutions at pH 7.0. An optimum concentration of iron ions was present in both the controls and manganese cultures. To cultures without manganese which had shown no growth for two weeks, manganese was added at the rate of 1 : 5,000,000. Growth then began and in two weeks there was about as much dry weight produced as in the manganese cultures at the end of the previous period. This is further evidence that manganese is required and also shows that the cells did not die but could not develop without manganese. Other experiments show that manganese will not replace iron in the nutrition of *Chlorella* and that a number of other elements will not replace manganese. The following elements were tested: Ba, Cu, Pb, Sr, Zn, B, Ni, I, As, Co, Al, all in concentrations of 1 : 5,000,000. The effect of increasing the concentration of manganese was studied. It was found that a concentration of 1 : 100,000 causes a marked depression in growth and 1 : 50,000 results in a definite toxicity. A hypothesis which has been developed for explaining the function of manganese is that it tends to control the ratio $[\text{Fe}^{++}] : [\text{Fe}^{+++}]$ in the culture solution or in the cell.

The Relative Effectiveness of the Temperature of the Crown as Contrasted with that of the Rest of the Plant upon the Flowering of Celery Plants. *Otis F. Curtis and H. T. Chang, Cornell University, Ithaca, N. Y.*

—A number of investigators have demonstrated that the flowering of several kinds of plants, notably celery and cabbage, can be induced by exposing the plants to a relatively low temperature, about 40°–60° F. (or 4°–15° C.), for a period of from one to several weeks whereas keeping the plants at a higher temperature, 70°–80° F. (or 21°–30° C.), may greatly delay or completely prevent this flowering. Garner and Allard have demonstrated that the effects of light duration on flowering are somewhat localized, while Knott has demonstrated that the effect of light duration on the flowering of cosmos seems to be limited to the growing tip or to the tissues within a millimeter or two of this tip. It seemed desirable to determine the effect of localized temperature differences upon the behavior of celery. The crown was therefore encased in coils of rubber tubing through which water at controlled temperatures could be forced. Under conditions where the crown was kept cool but the rest of the plant was in a warm house the plant flowered as if the entire plant had been in a cool house, whereas when the plants were kept in a cool house, where they would normally develop flower stalks, they failed to do this when the crowns were kept at the higher temperatures. These findings should have a bearing on the question as to what tissues should be selected for analysis in attempts to determine the reasons for the influence of various factors on fruiting.

The Influence of Various Types of Defoliation and Leaf Wounding upon the Growth and Yield of Beans. *H. L. Chance, Cornell University, Ithaca, N. Y.*—The purpose of the work was to determine the influence of various types of leaf wounding, as carried out upon the first pair of leaves of beans, upon the subsequent growth and yield of leaves, fruit, stems, and roots. The wounding consisted of the removal of one-half or one-fourth of the total leaf area either en bloc or in the form of disks. Another method consisted of slashing the blades without the removal of tissue. The disks were removed to simulate insect injury, while the slashing is, in a measure, comparable to hail injury. The effect of the various types of wounding upon the number of flowers and the time of blooming period was also studied. Plants were harvested at four different stages of development, namely: at the appearance of the first flower, just prior to full bloom, just after full bloom, and after the pods were well-formed. When harvested after the pods were well-formed, there was no significant difference between the yield of any part studied and that of the corresponding part of the check, though there was a slight increase in the yield of leaves and a slight decrease in the yield of roots. Earlier harvests generally gave a lower yield for each part studied. While the number of flowers was not reduced, the blooming period was slightly delayed.

Estrogenic Substances in Growing Plants. *Burnham S. Walker, Evans Memorial and Boston University School of Medicine, Boston, Mass.*—Pre-

vious workers have demonstrated that certain plant extracts have an action similar to the "female sex hormone" of the animal kingdom, in that they are capable of initiating estrus in castrated female animals. The present report deals with the investigation of a number of plants and plant constituents for estrogenic activity. The majority of the extracts yielded negative results; positive results were obtained from extracts of green parts of plants, collected and extracted during the period of rapid growth.

Relation between Time of Exposure of Orchid Seed to Sugar, and Seedling Development. *Lewis Knudson and Daniel G. Clark, Cornell University, Ithaca, N. Y.*—In previous work on the germination of orchid seed Knudson proposed the view that the failure of orchid seed germination when the seed are supplied with nutrients, water, and favorable growth conditions, is lack of adequate organic food material. When the orchid fungus is supplied to a medium containing starch the fungus digests the starch and thus sugar is made available. From earlier studies it was evident that the embryo could be germinated with an appropriate medium containing an available sugar and then transplanted to a purely inorganic medium on which it would develop. This implies that the embryo must be supplied with sugar for a definite period in its early existence. The present experiments were devised for this purpose. Seed were sown in tubes containing a nutrient medium with sugar. Controls were prepared lacking sugar. From the sugar medium seed were transferred to a purely inorganic medium after 1 day, 2, 3, 4, 6, 8, 12, 16, and 20 days. Other seed were maintained on the sugar medium throughout the period of the experiment. Four percent of the seed on the purely inorganic medium developed into seedlings. With sugar all of the seed developed into seedlings. No significant difference in growth of embryos occurred with the various treatments until the seed had been left on the sugar medium for from 8 to 12 days. With these treatments the percentage of seedlings produced was 8 percent and 11 percent, respectively. With 20 days exposure to sugar the percentage of seedlings produced was only 11 percent. If delayed photosynthesis is the cause of the failure of seedling production, the retardation of photosynthesis for most of the embryos under the conditions of this experiment is more than 20 days.

Direct vs. Indirect Effects in the Treatment of Potato Tubers with Chemicals. *F. E. Denny, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—When dormant potato tubers are treated with either sodium thiocyanate or ethylene chlorhydrin starch decreases and cane sugar increases more rapidly in treated than in check lots. If the breakdown of starch is brought about by the enzyme amylase we should expect the amylase activity of the juice from treated lots to be higher than that from the checks. This has been found to be the case, the gain of treated

over check being greater for ethylene chlorhydrin than for sodium thiocyanate. In order to test whether this is an indirect effect or a direct effect of the chemical upon the amylase, the chemicals were added to potato juice and the gain in reducing sugar resulting from contact with starch solution was measured. At the pH of potato juice (usually 6.0-6.3) both ethylene chlorhydrin and sodium thiocyanate decrease the amylase activity of potato juice. About 20 milligrams of NaSCN in 100 cc. of reacting mixture of juice and starch solution definitely depressed the action and 100 milligrams gave strong retardation. Under the same conditions decreases were observed with amounts of ethylene chlorhydrin in the range from 0.5 cc. to 2.5 cc. of 40 percent ethylene chlorhydrin. Thus, although treating potato tissue with the chemicals caused an increase in amylase activity, treating the juice caused a decrease; it is concluded that the effect of the chemicals in increasing the amylase activity of the tissue was indirect, and was not due to any direct stimulative influence of the chemical upon the enzym.

Increasing Amylase Activity of Potato Juice by the Addition of Potassium Cyanid. *F. E. Denny, Boyce Thompson Institute for Plant Research, Yonkers, N. Y.*—At the 1930 meeting of the International Botanical Congress at Cambridge, Hanes and Barker showed that cyanid increased the activity of malt amylase, and this fact was suggested as an explanation of the effect of HCN in increasing the respiration of potato tubers. However, they did not determine the effect of cyanid upon potato amylase and the experiments here reported upon in a preliminary manner were carried out to test this point. Potassium cyanid added to a mixture of potato juice and soluble starch increased markedly the amylase activity as measured by the gain in reducing sugar; as little as 10 milligrams per 100 cc. of the reacting mixture increased the activity about 50 percent and 40 milligrams approximately doubled it. The effect of KCN in decreasing the hydrogen-ion concentration was overcome by the addition of the proper amount of acid to give the same pH as the water check. Hanes and Barker found that at ordinary temperatures the stimulating effect of cyanid upon malt amylase occurred only in the first few minutes, after which a depression was found. In these experiments with potato amylase increases were observed when the reaction had proceeded for 2 to 4 days at 35° C.

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